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Remarks:

The applicant has subsequently filed a sequence listing and declared, that it includes no new matter.

(54) Osteoclastgenic inhibitory agent comprising interleukin-18

(57) An osteoclastgenic inhibitory agent which comprises an interleukin-18 and/or its functional equivalent. The agent can be arbitrarily used as an ingredient for cell culture and agents for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Description

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The present invention relates to an osteoclastgenic inhibitory agent comprising an interleukin-18 (hereinafter abbreviated as "IL-18") or its functional equivalent.

Osteoblasts' bone formation and osteoclasts' bone resorption are well balanced in healthy living bodies, and this keeps the bone tissues in normal conditions while old bone tissues are being replaced with fresh ones without altering the original bone shape. The phenomenon plays an important role in keeping living bodies' homeostasis such as the controlling of blood calcium concentration within a desired range. Once the balance is lost, especially when the bone resorption level exceeds the bone formation level, bone-related diseases and other diseases may be induced. Therefore, elucidation of the whole mechanism of bone resorption in living bodies, particularly, elucidation of osteoclasts is greatly highlighted due to scientific and clinical significance thereof.

However, the mechanism of osteoclast formation has not yet been completely elucidated even though interleukin 1 as a promoter and interleukin 4 as an inhibitor were found. This is because, similarly as various phenomena in living bodies, osteoclast formation in living bodies is controlled by the close and complicated relationship between promoters and inhibitors. Based on these, it is greatly expected to establish an effective osteoclastgenic inhibitory agent from the viewpoint of scientific and clinical aspects.

The object of the present invention is to provide a novel and effective osteoclastgenic inhibitory agent. To solve the object the present inventors energetically studied for IL-18, i.e., one of cytokines as communication transferring substances in immune systems, which induces production of interferon-y (hereinafter abbreviated as "IFN-γ"), an important biologically active substance for immunocompetent cells, and granulocyte/macrophage colony-stimulating factor (hereinafter abbreviated as "GM-CSF"), and augments cytotoxicity and induces formation of killer cells. At the finding, IL-18 was described as an interferon-γ-inducing factor as reported by Haruki OKAMURA in Japanese Patent Kokai Nos. 27,189/96 and 193,098/96, and in *Nature*, Vol. 378, No. 6,552, pp. 88-91 (1995), and then called IL-18 according to the proposal of Shimpei USHIO et al., in *The Journal of Immunology*, Vol. 156, pp. 4,274-4,279 (1996).

The present inventors found that a particular gene, capable of inhibiting osteoclast formation from osteoclastic precursor cells *in vitro*, is specifically expressed in quantities in stroma cells derived from mouse myeloma. Their further detailed analysis revealed that (i) the gene encodes IL-18 that includes SEQ ID NO: 7 as a core sequence, (ii) IL-18 and functional equivalents thereof effectively inhibit osteoclast formation, and (iii) the inhibition is mainly due to the action of GM-CSF induced and produced by IL-18.

Based on these, the present inventors solved the present object by an osteoclastgenic inhibitory agent comprising IL-18 or its functional equivalent as an effective ingredient.

FIG. 1 shows the structure of the recombinant DNA pKGFHH2.

FIG. 2 shows the structure of the recombinant DNA pCSHIGIF/MUT35.

FIG. 3 shows the structure of the recombinant DNA pCSHIGIF/MUT42.

FIG. 4 shows the structure of the recombinant DNA pBGHuGF.

FIG. 5 shows the structure of the recombinant DNA pKGFMH2.

In these figures, KGFHH2 cDNA means a cDNA encoding the IL-18 according to the present invention: IGIF/MUT35; a DNA encoding the IL-18 according to the present invention: IGIF/MUT42; a DNA encoding the IL-18 according to the present invention: HulGIF; a chromosomal DNA encoding the IL-18 according to the present invention: KGFMH2 cDNA; a cDNA encoding the IL-18 according to the present invention: 5S; a gene for 5S ribosomal RNA: Ptac; a tac promoter: rrnBT1T2; a termination region of a ribosomal RNA operon: AmpR; an ampicillin resistent gene: pBR322ori; a replication origin of *Escherichia coli*: CMV; a cytomegalovirus promoter: IFNss; a nucleotide sequence encoding a signal peptide for subtype α2b of human interferon-α.

The present invention relates to an osteoclastgenic inhibitory agent comprising IL-18 or its functional equivalent as an effective ingredient. The wording "IL-18" as referred to in the invention includes polypeptides with the above property independently of their sources and origins. For example, the IL-18 used in the present invention includes, as internal partial amino acid sequences, the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, as well as SEQ ID NO: 4 and SEQ ID NO: 5, and includes the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 7 as a whole. The wording "functional equivalent(s)" as referred to in the present invention includes (i) those wherein one or more amino acids in the amino acid sequence of IL-18 are replaced with different amino acids, (ii) those wherein one or more amino acids are added to the N- and/or C-terminal regions of the amino acid sequence of IL-18, (ivi) those wherein one or more amino acids in the N- and/or C-terminal regions of the amino acid sequence of IL-18 are deleted, and (v) those wherein one or more amino acids in the internal regions of the amino acid sequence of IL-18 are deleted, all of these modifications should be made within the range that does not substantially lose the property of osteoclast formation by IL-18 among the inherent property of IL-18. Examples of such functional equivalents are described along with their detailed amino acid sequences in Japanese Patent Application No. 20,906/97 by the same applicant of the present applicant, i.e., polypeptides which are capable of inducing production of interferon-gamma by immunocompe-

tent cells, wherein said polypeptides contain either amino acid sequence wherein one or more cysteines are replaced with different amino acid(s) while leaving respective consensus sequences as shown in SEQ ID NOs: 1, 2 and 4 intact, or that wherein one or more amino acids are added, removed and/or replaced at one or more sites including those in the consensus sequences but excluding those of the replaced cysteine. The different amino acids to replace the cysteine(s) are not restricted to any types, as far as the resulting polypeptide, containing an amino acid sequence replaced with the different amino acid(s), exhibits an activity of inducing production of IFN-γ by immunocompetent cells in the presence or absence of an appropriate cofactor, as the wild-type polypeptides containing SEQ ID NOs: 1, 2 and 4 as consensus partial amino acid sequences, and a stability significantly higher than that of the wild-type polypeptides. The different amino acids include serine, threonine, alanine, valine, leucine, isoleucine, histidine, tyrosine, phenylalanine, tryptophan, and methionine, among which the most preferable amino acid is serine or alanine. Embodiments of the amino acid sequences, containing SEQ ID NOs: 1, 2 and 4 as consensus partial amino acid sequences, in which one or more cysteines are to be replaced with different amino acid(s) are the wild-type polypeptides containing SEQ ID NO: 6 or 7. SEQ ID NO: 6 contains cysteines at the 38th, 68th, 76th, and 127th positions from the N-terminus. SEQ ID NO: 7 contains cysteines at the 7th, 75th, and 125th positions. The polypeptides include those containing the amino acid sequence of any one of SEQID NOs: 20-26, which are derived from the wild-type polypeptide containing SEQID NO: 6, those containing the amino acid sequence of SEQ ID NO: 27 or 28, which are derived from the wild-type polypeptide containing the amino acid sequence of SEQ ID NO: 7, and those containing an amino acid sequence derived from any one of SEQ ID NOs: 20-28 by adding, removing, and/or replacing one or more amino acids to and/ or at position(s) excepting the positions where the cysteine(s) have been replaced while retaining the desired biological activities and stability. The wording "one or more amino acids" means the number of amino acids which conventional methods such as site-directed mutagenesis can usually add, remove or replace. The polypeptides containing any one of SEQ ID NOs: 20-28 possess both stability and biological activities significantly higher than those of the wild-type polypeptides.

The functional equivalents as referred to in the present invention further include glycosylated polypeptides of IL-18 and the above polypeptides. Any of these IL-18 and functional equivalents thereof, both of which are included to and referred to as "IL-18" in the present invention, unless specified otherwise, can be used in the present invention independently of their origins; those prepared by separating from natural sources such as cell cultures and from artificially synthesized ones using recombinant DNA technology and peptide synthesis.

With economical viewpoint, methods of recombinant DNA technology are advantageously used; generally, desired IL-18 can be obtained by introducing DNAs encoding IL-18 into appropriate hosts derived from microorganisms, plants, and animals to form transformants, culturing the transformants in nutrient culture media in a conventional manner, and purifying the cultures by conventional methods used for purifying cytokines. Any DNAs can be used as the above DNAs as long as they contain a DNA encoding IL-18, and can be suitably selected depending on the purpose of the use of the present osteoclastgenic inhibitory agent or on the recombinant DNA technology used. For example, Japanese Patent Kokai Nos. 193,098/96, 231,598/96, and 27,189/96 by the same applicant of the present invention disclose in detail methods for producing IL-18 by culturing transformed microorganisms into which DNAs including a cDNA encoding mouse or human IL-18 are introduced; and Japanese Patent Application No. 185,305/96 by the same applicant of the present invention discloses in detail a method for producing IL-18 encoding human IL-18 by culturing transformed animal cells which have an introduced DNA that includes a chromosomal DNA encodes human IL-18. Japanese Patent Application No. 20,906/97 by the same applicant of the present invention discloses in detail a method for producing IL-18 by culturing transformed animal cells having an introduced DNA which includes a DNA encoding a functional equivalent of human IL-18.

The aforesaid recombinant DNA technology has an economical advantage, but depending on the hosts and DNA sequences used, the IL-18 thus obtained may have somewhat different physicochemical property from those of IL-18 produced and functions *in vivo*. Japanese Patent Application No. 67,434/96 by the same applicant of the present invention discloses in detail a preparation of IL-18 using established human cell lines as natural sources, and Japanese Patent Application No. 213,267/96 by the same applicant also discloses in detail the preparation using an interleukin-1β-converting enzyme. The IL-18 obtained by those preparations can be estimated to have substantially the same or equal physicochemical property to that of IL-18 that is produced and functions in *vivo*, and the yield can be estimated to be slightly lower. However, such IL-18 has an advantage that it has a fewer side effects when used as pharmaceuticals directed to administering to warm-blooded animals in general and including humans. When applying purification methods using monoclonal antibodies specific to IL-18, as disclosed in Japanese Patent Application No. 231,598/96 by the same applicant of the present invention, a relatively-high purity IL-18 can be obtained in a minimum labor and cost.

The present osteoclastgenic inhibitory agent comprising the aforesaid IL-18 includes any types and forms usable to inhibit osteoclast formation both in vivo and in vitro. The present agent can be advantageously used as ingredients for cell culture media for animal cells, which satisfactorily inhibit osteoclast formation, maintain, proliferate, and/or differentiate the desired cells; components of screening kits for bone-related therapeutic agents; bone-resorption regulatory agents for osteoclast-related diseases. The bone-resorption regulatory agents include medica-

ments and health foods that exert an osteoclastgenic inhibitory activity in *vivo*, control bone resorption to normal conditions, and improve unfavorable physical conditions such as a relatively-insignificant arthralgia. The agents for osteoclast-related diseases include medicaments used to prevent and/or treat diseases caused by an excessive osteoclast formation and/or its function. Examples of such diseases are hypercalcemia, osteoclastoma, Behcet's syndrome, osteosarcoma, arthropathy, chronic rheumatoid arthritis, deformity ostitis, primary hyperthyroidism, osteopenia, and osteoporosis. Varying depending on the types of agents and diseases to be treated, the present agent is usually formulated into a liquid, paste, or solid form which contains 0.000002-100 w/w %, preferably, 0.0002-0.5 w/w % of IL-18.

The present osteoclastgenic inhibitory agent can be IL-18 alone or compositions comprising IL-18 and one or more other ingredients such as carriers, excipients, diluents, adjuvants, antibiotics, and proteins such as serum albumin and gelatin as stabilizers; saccharides such as glucose, maltose, maltotriose, maltotraose, trehalose, sucrose, isomaltose, lactose, panose, erlose, palatinose, lactosucrose, raffinose, fructooligosaccharide, galactooligosaccharide, lentinan, dextrin, pullulan, and sugar alcohols including sorbitol, maltitol, lactitol, and maltotriitol; buffers comprising phosphates or citrates mainly; and reductants such as 2-mercaptoethanol, dithiothreitol, and reduced glutathione; and optionally biologically active substances such as interferon-a, interferon-p, interferon-γ, interleukin-2, interleukin-3, interleukin-6, interleukin-12, TNF-α, TNF-β, GM-CSF, estrogen, progesterone, chlormadinone acetate, calcitonin, somatokine, somatomedin, insulin-like growth factor, ipriflavone, parathyroid hormone (PTH), norethisterone, busulfan, ancitabine, cytarabine, fluorouracil, tetrahydrofurfuryl fluorouracil, methotrexate, vitamin D₂, active vitamin D, Krestin® or polysaccharide K, L-asparaginase, and OK-432 or Picibanil®; and calcium salts such as calcium lactate, calcium chloride, calcium monohydrogenphosphate, and L-calcium L-aspartate. When used as agents for administering to warmblooded animals in general and including humans, i.e., agents for osteoclast-related diseases, the present agent can be preferably formulated into compositions by appropriately combining with one or more of the above physiologically-acceptable substances.

The present osteoclastgenic inhibitory agent includes medicaments in a unit dose form used for administering to warm-blooded animals in general and including humans. The wording "unit dose form" means those which contain IL-18 in an amount suitable for a daily dose or in an amount up to four fold by integers or up to 1/40 fold of the dose, and those in a physically separated and formulated form suitable for prescribed administrations. Examples of such formulations are injections, liquids, powders, granules, tablets, capsules, troches, collyriums, nebulas, and suppositories.

The present agent as an osteoclastgenic inhibitory agent effectively treat and prevent osteoclast-related diseases independently of oral and parenteral administrations. Varying depending on the types and symptoms of patients' diseases, the present agent can be administered to the patients orally, intradermally, subcutaneously, muscularly, or intravenously at a dose of about 0.5 µg to 100 mg per shot, preferably, at a dose of about 2 µg to 10 mg per shot of IL-18, 2-6 fold a day or 2-10 fold a week for one day to one year.

In the below, with reference to experiments, the preparation, physicochemical property, and biological activity of the IL-18 according to the present invention are described:

Experiment 1

Preparation of human IL-18

According to the method in Japanese Patent Kokai No. 231,598/96 by the same applicant of the present invention, an autonomously-replicable recombinant DNA, pKGFHH2, linked to a cDNA encoding human IL-18, was prepared. Dideoxyribonucleotide sequencing analyzed that, as shown in FIG. 1, in the recombinant DNA, KGFHH2 cDNA containing the base sequence of SEQ ID NO: 8 was linked to the downstream of Ptac, a Tac promoter. The recombinant DNA pKGFHH2 contained the amino acid sequences of SEQ ID NOs: 1 to 5; these amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 8.

According to the method in Japanese Patent Kokai No. 231,598/96, the recombinant DNA pKGFHH2 was introduced into an *Escherichia coli* Y1090 strain, ATCC 37197, and the strain was cultured. The produced polypeptide was purified by immunoaffinity chromatography to obtain a purified human IL-18 with a purity of at least 95% in a yield of about 25 mg/ℓ culture. According to the method in Japanese Patent Kokai No. 193,098/96 by the same applicant of the present invention, the purified human IL-18 was analyzed for biological activity and physicochemical property as indicated below: When culturing human lymphocytes, collected by a conventional manner from a healthy donor, in the presence of the purified human IL-18, IFN-γ production was observed depending on the concentration of IL-18, resulting in a confirmation that IL-18 has an activity of inducing IFN-γ production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified IL-18 was subjected to SDS-PAGE, resulting in a major band with an IFN-γ inducing activity at a position corresponding to 18,500±3,000 daltons. The IL-18 gave a pl of 4.9±1.0 as determined by conventional chromatofocusing. Conventional analysis using "PROTEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City,

USA, revealed that the IL-18 had the amino acid sequence of SEQ ID NO: 9, i.e., the amino acid sequence of SEQ ID NO: 8 where a methionine residue was linked to the N-terminus.

Experiment 2

Preparation of human IL-18

According to the method in Japanese Patent Application No. 67,434/96 by the same applicant of the present invention, THP-1 cells, ATCC TIB 202, a human monocyte cell line derived from a male with acute monocytic leukemia, were inoculated to the dorsum subcutaneous tissues of new born hamsters, followed by feeding the hamsters for three weeks. Tumor masses, about 15 g weight each, formed in the subcutaneous tissues of each hamster, were extracted, dispersed in media, and disrupted. The polypeptide obtained from the disrupted cells was purified by immunoaffinity chromatography to obtain a purified human IL-18 in a yield of an about 50 ng/head.

Similarly, according to the method in Japanese Patent Application No. 67,434/96, the purified human IL-18 was analyzed and determined for biological activity and physicochemical property as indicated below: It was confirmed that culturing human lymphocytes, collected from healthy donors in a conventional manner, in the presence of different concentrations of the human IL-18, resulted in an IL-18 dose-dependent IFN-y production. This revealed that the human IL-18 has a biological activity of inducing IFN-y production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in Nature, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE using 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN-y production inducing activity at a position corresponding to 18,000-19,500 daltons. According to the peptide map disclosed in Japanese Patent Application No. 67,434/96, the human IL-18 was treated with clostripain commercialized by Sigma Chemical Company, Missouri, USA, to obtain polypeptide fragments, followed by subjecting the fragments for fractionation to high-performance liquid chromatography (HPLC) using "ODS-120T", a column commercialized by Tosoh Corporation, Tokyo, Japan, and analyzing the amino acid sequences of the fragments from the N-terminus to reveal the following amino acid sequences of SEQ ID NOs: 10 to 13. These amino acid sequences were completely coincided with amino acids 148-157, 1-13, 45-58, and 80-96 in SEQ ID NO: 6. The data shows that the human IL-18 obtained in Experiment 2 has the amino acid sequence of SEQ ID NO: 6 and all the partial amino acid sequences of SEQ ID NOs: 1 to 5.

Experiment 3

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Preparation of functional equivalents

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, it was prepared an autonomously-replicable recombinant DNA, **pCSHIGIF/MUT35**, was linked to a DNA encoding a functional equivalent of human IL-18 where cysteines 38, 68, and 76 in SEQ ID NO: 6 were respectively replaced with serine, serine, and alanine. Dideoxyribonucleotide sequence analysis revealed that as shown in FIG. 2, in the recombinant DNA, DNA IGIF/MUT35 with SEQ ID NO: 14 linked to the downstream of a base sequence encoding a signal peptide of subtype α2b in human interferon-a in the same reading-frame, as reported by K. Henco et al., in *Journal of Molecular Biology,* Vol. 185, pp. 227-260 (1985), and had a stop codon for protein synthesis at further downstream. As shown in parallel in SEQ ID NO: 14, the amino acid sequence encoded by the recombinant DNA corresponded to SEQ ID NO: 6 where cysteines 38, 68, and 76 in SEQ ID NO: 6 were respectively replaced with serine, and alanine. The recombinant DNA contained a nucleotide which encodes all the amino acid sequences of SEQ ID NOs: 1 to 4 and the one of SEQ ID NO: 5 where cysteine at amino acid 5 in SEQ ID NO: 5 was replaced with alanine. These amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 14.

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, the recombinant DNA pCSHIGIF/MUT35 was introduced into COS-1 cells, ATCC CRL 1650, an established cell line derived from SV40 transformed African Green monkey kidney, followed by culturing the transformed cells. The produced polypeptide in the culture was purified by immunoaffinity chromatography to obtain a purified functional equivalent of human IL-18 in a yield of about 40 ng/ml culture. According to the method in Japanese Patent Application No. 20,906/97, the purified functional equivalent was analyzed and determined for biological activity and physicochemical property as indicated below: When culturing KG-1 cells, ATCC CCL 246, an established cell line derived from human acute myelogenous leukemia, in the presence of different concentrations of the purified functional equivalent of human IL-18, IFN-y production was observed depending on the concentration of the IL-18, revealing that the IL-18 has a biological activity of inducing IFN-y production by KG-1 cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified functional equivalent was

subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN-γ production inducing activity at a position corresponding to 18,000-19,500 daltons. Conventional analysis using "PRO-TEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City, USA, revealed that the N-terminal region of the functional equivalent had the amino acid sequence of SEQ ID NO: 15 which corresponded to the amino acid sequence in the N-terminal region as shown in parallel in SEQ ID NO: 14.

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present

Experiment 4

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Preparation of functional equivalent

invention, it was prepared an autonomously-replicable recombinant DNA, pCSHIGIF/MUT42, which was linked to a DNA encoding for a functional equivalent of human IL-18 where cysteines 38, 68, 76, and 127 in SEQ ID NO: 6 were respectively replaced with serine, serine, alanine, and serine. Dideoxyribonucleotide sequencing revealed that, as shown in FIG. 3, in the recombinant DNA, DNA IGIF/MUT42 with SEQ ID NO: 16 linked to the downstream of a base sequence encoding a signal peptide for subtype o2b of human interferon-a in the same reading frame, as reported by K. Hence et al., in *Journal of Molecular Biology*, Vol. 185, pp. 227-260 (1985), and had a stop codon for protein synthesis

K. Henco et al., in *Journal of Molecular Biology*, Vol. 185, pp. 227-260 (1985), and had a stop codon for protein synthesis at further downstream. As shown in parallel in SEQ ID NO: 16, the amino acid sequence encoded by the recombinant DNA corresponded to SEQ ID NO: 6 where cysteines 38, 68, 76, and 127 in SEQ ID NO: 6 were respectively replaced with serine, serine, alanine, and serine. The recombinant DNA contained a nucleotide sequence which encodes all the amino acid sequences of SEQ ID NOs: 1 to 4 and the one of SEQ ID NO: 5 where cysteine 5 in SEQ ID NO: 5 was replaced with alanine. These amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 16.

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, the recombinant DNA pCSHIGIF/MUT42 was introduced into COS-1 cells, followed by culturing the cells. The produced polypeptide in the culture was purified by immunoaffinity chromatography to obtain a purified functional equivalent of human IL-18 in a yield of about 20 ng/ml culture. According to the method in Japanese Patent Application No. 20,906/97, the purified functional equivalent was analyzed and determined for biological activity and physicochemical property as indicated below: When cultured KG-1 cells in the presence of different concentrations of the purified functional equivalent, a dose-dependent IFN-γproduction was observed, and this revealed that the functional equivalent has a biological activity of inducing IFN-γ production by KG-1 cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified functional equivalent was subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN-γ inducing activity at a position corresponding to 18,000-19,500 daltons. Conventional analysis using "PROTEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City, USA, revealed that the N-terminal region of the functional equivalent had the amino acid sequence of SEQ ID NO: 15 which completely corresponded to the amino acid sequence in the N-terminal region as shown in parallel in SEQ ID NO: 16.

Experiment 5

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Preparation of human IL-18

According to the method in Japanese Patent Application No. 185,305/96 by the same applicant of the present invention, an autonomously-replicable recombinant DNA, pBGHuGF, linked to a chromosomal DNA encoding human IL-18, was obtained. Dideoxyribonucleotide sequencing analysis revealed that as shown in FIG. 4, in the recombinant DNA, a chromosomal DNA, which encodes human IL-18, i.e., DNA HulGIF with SEQ ID NO: 17, was linked to the downstream of a restriction site by a restriction enzyme, *Hind* III. As shown in SEQ ID NO: 17, the chromosomal DNA HulGIF consists of 11,464 bp where the exon was fragmented by four introns positioning at nucleotides 83-1,453, 1,466-4,848, 4,984-6,317, and 6,452-11,224. Among the resting nucleotide sequence excluding these introns, nucleotides 3-11,443 from the 5'-terminus are the part that encodes a precursor of human IL-18, and nucleotides 4,866-4,983 are the part that encodes an active human IL-18. The chromosomal DNA contained nucleotides sequences encoding SEQ ID NOs: 1 to 5; these amino acid sequences were respectively encoded by nucleotides 4,911-4,928, 4,953-4,970, 11,372-11,392, 6,350-6,364, and 6,413-6,427 in SEQ ID NO: 17.

According to the method in Japanese Patent Application No. 185,305/96, the recombinant DNA pBGHuGF was introduced into CHO-K1 cells, ATCC CCL 61, an established cell line derived from Chinese hamster ovary, followed by culturing the cells. The culture supernatant was contacted with a supernatant of cell disruptant prepared from a THP-1 cell culture to produce a polypeptide which was then purified by immunoaffinity chromatography to obtain a purified human IL-18 in a yield of about 15 mg/ ℓ culture. According to the method in Japanese Patent Application No.

185,305/96, the polypeptide was analyzed and determined for biological activity and physicochemical property as indicated below: It was confirmed that human lymphocytes, which were collected from a healthy donor, produced IFN-γ depending on the purified human IL-18 concentration when cultured at different concentrations of the human IL-18, revealing that the human IL-18 has a biological activity of inducing IFN-γ production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN-γ inducing activity at a position corresponding to 18,000-19,500 daltons. The N-terminal region of the human IL-18 contained the amino acid sequence of SEQ ID NO: 15 which completely corresponded to the amino acid sequence in the N-terminal region of SEQ ID NO: 17 for an active IL-18. Experiment 6

Preparation of mouse IL-18

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To a 0.5-ml reaction tube were added 8 μ l of 25 mM magnesium chloride, 10 μ l of 10 x PCR buffer, one μ l of 25 mM dNTP mix, one μ l of 2.5 units/ μ l of amplitaq DNA polymerase, one ng of a recombinant DNA, which encodes mouse IL-18 having the nucleotide sequence of SEQ ID NO: 18 and the amino acid sequence of SEQ ID NO: 7, prepared from a phage DNA clone according to the method in Japanese Patent Kokai No. 27,189/96, and adequate amounts of a sense and antisense primers having nucleotide sequences represented by 5'-ATAGAATTCAAAT-GAACTTTGGCCGACTTCACTG-3' and 5'-ATAAAGCTTCTAACTTTGATGTAAGTT-3', respectively, which were chemically synthesized based on the amino acid sequences nearness to the N- and C-termini of SEQ ID NO: 7, and the mixture solution was brought up to a volume of 100 μ l with sterilized distilled water. The solution thus obtained was subjected in a usual manner to PCR reaction of the following three cycles of successive incubations at 94°C for one minute, 43°C for one minute, and 72°C for one minute, and 72°C for one minute.

The product obtained by the PCR reaction and "pCR-Script SK (+)", a plasmid vector commercialized by Stratagene Cloning Systems, California, USA, were in a conventional manner ligated together using a DNA ligase into a recombinant DNA which was then introduced into "XL-1 Blue MRF'Kan", an Escherichia coli strain commercialized by Stratagene Cloning Systems, California, USA., to obtain a transformant. The transformant was inoculated to L-broth (pH 7.2) containing 50 µg/ml ampicillin, followed by the incubation at 37°C for 18 hours under shaking conditions. The culture was centrifuged to obtain the proliferated transformants which were then treated with a conventional alkali-SDS method to isolate a recombinant DNA. A portion of the recombinant DNA isolated was analyzed by dideoxyribonucle-otide sequencing, revealing that the recombinant DNA contained restriction sites of Eco RI and Hind III at the 5'- and 3'-termini of SEQ ID NO: 18, respectively, and a DNA containing a methionine codon for initiating polypeptide synthesis and a TAG codon for terminating polypeptide synthesis, which were located in just before and after the N- and C-termini of the amino acid sequence as shown in parallel in SEQ ID NO: 18. The recombinant DNA contained the nucleotide sequences of SEQ ID NOs: 1 to 5. These amino acid sequences were encoded by nucleotides 46-63, 85-102, 394-414, 148-162, and 211-225 in SEQ ID NO: 18.

The remaining portion of the recombinant DNA was in a conventional manner cleaved with restriction enzymes of *Eco* RI and *Hind* II, and the resulting 0.1 μg of an *Eco RI-Hind* III DNA fragments, obtained by using "DNA LIGATION KIT VER 2", a DNA ligation kit commercialized by Takara Shuzo Co., Ltd., Tokyo, Japan, and 10 ng of pKK223-3, a plasmid vector commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been cleaved with a restriction enzyme were linked together, by incubating at 16°C for 30 min to obtain an autonomously-replicable recombinant DNA, pKGFMH2. Using competent cell method, an *Escherichia coli* Y1090 strain, ATCC 37197, was transformed using the recombinant DNA pKGFMH2, and the resulting transformant, KGFMH2, was inoculated to L-broth (pH 7.2) containing 50 μg/ml ampicillin, and cultured at 37°C for 18 hours under shaking conditions. The culture was centrifuged to collect the proliferated transformants, followed by applying a conventional SDS-alkali method to a portion of the transformants to extract the recombinant DNA pKGFMH2. Dideoxyribonucleotide sequencing analysis revealed that, as shown in FIG. 5, KGFMH2 cDNA containing the nucleotide sequence of SEQ ID NO: 18 was linked to the downstream of the Tac promoter in the recombinant DNA pKGFMH2.

Ampicillin was added to L-broth (pH 7.2), which had been sterilized by autoclaving, to give a concentration of 50 μg/ml, cooled to 37°C, and inoculated with the transformant KGFMH2, followed by the culture at 37°C for 18 hours. Eighteen liters of a fresh preparation of the same culture medium was placed in a 20-ℓ jar fermenter, similarly sterilized as above, admixed with ampicillin, cooled to 37°C, and inoculated with one v/v % of the seed culture obtained in the above, followed by the culture at 37°C for 8 hours under aeration-agitation conditions. The resulting culture was centrifuged to collect the cultured cells which were then suspended in a mixture solution (pH 7.3) containing 150 mM sodium chloride, 16 mM disodium hydrogenphosphate, and 4 mM sodium dihydrogenphosphate, disrupted by ultrasonication, and centrifuged to remove cell disruptant, and this yielded an about two liters of a supernatant.

To an about two liters of the supernatant was added 10 mM phosphate buffer (pH 7.3) containing ammonium sulfate to give a 40% ammonium saturation. The resulting sediment was removed by centrifugation, and the supernatant

was mixed with ammonium sulfate to give an 85% ammonium saturation, allowed to stand at 4°C for 18 hours, and centrifuged at about 8,000 rpm for 30 min to obtain a newly formed sediment. The sediment thus obtained was dissolved in 10 mM phosphate buffer (pH 6.6) containing 1.5 M ammonium sulfate to give a total volume of about 1,300 ml, and the solution was filtered, and fed to a column packed with about 800 ml of "PHENYL SEPHAROSE CL-6B", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, followed by washing the column with a fresh preparation of the same buffer and feeding to the column a linear gradient buffer of ammonium sulfate decreasing from 1.5 M to O M in 10 mM phosphate buffer (pH 6.6) at an SV (space velocity) 1.5. Fractions eluted at around 1 M ammonium sulfate were pooled, concentrated using a membrane filter, and dialyzed against 10 mM phosphate buffer (pH 6.5) at 4°C for 18 hours. The dialyzed solution was fed to a column packed with about 55 ml of "DEAE-5PW", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been equilibrated with 10 mM phosphate buffer (pH 6.5). The column was washed with a fresh preparation of the same buffer, and fed with a linear gradient buffer of sodium chloride increasing from 0 M to 0.5 M in 10 mM phosphate buffer (pH 6.5) at SV 5.5, followed by collecting fractions eluted at around 0.2 M sodium chloride. Thereafter, the fractions were pooled and concentrated similarly as above up to give an about nine milliliters, followed by dialyzing the concentrate against PBS (phosphate buffered saline) at 4°C for 18 hours, and feeding the dialyzed solution to a column packed with "SUPERDEX 75", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been equilibrated with a fresh preparation of the same PBS. The column was fed with a fresh preparation of the same PBS to collect fractions with an IFN-y inducing activity, and the fractions were pooled and concentrated with a membrane filter to obtain a purified mouse IL-18 in a yield of about 350 μg/ℓ culture.

According to the method in Japanese Patent Kokai No. 27,189/96, the purified mouse IL-18 was analyzed and determined for biological activity and physicochemical property as indicated below: Culturing mouse spleen cells, collected by a conventional manner, under different concentrations of the mouse IL-18 resulted in an IFN-γ production depending on the concentrations of the mouse IL-18, and this revealed that the mouse IL-18 has an activity of inducing IFN-γ production by spleen cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE under non-reducing conditions, resulting in a major band with an IFN-γ inducing activity at a position corresponding to 19,000±5,000 daltons. The N-terminal region of the mouse IL-18 contained the amino acid sequence of SEQ ID NO: 19 which corresponded to the N-terminal region of SEQ ID NO: 18.

With reference to Experiment 7, the biological activity of the IL-18 according to the present invention will be described in more detail, and Experiment 8 describes the cytotoxicity of the IL-18:

Experiment 7

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Biological activity

Experiment 7-1

Induction of GM-CSF production

Using a heparinized syringe, blood was collected from a healthy volunteer and diluted two fold with serum-free RPMI 1640 medium (pH 7.4). The diluent was overlaid on a ficoll and centrifuged, and the collected lymphocytes were washed with RPMI 1640 medium (pH 7.4) supplemented with 10 v/v % fetal calf serum, and suspended in a fresh preparation of the same medium to give a cell density of 1 x 10⁶ cells/mI, followed by distributing the cell suspension to a 12-well microplate by two mI/well.

Using RPMI 1640 medium (pH 7.4) supplemented with 10 v/v % fetal calf serum, an IL-18 preparation obtained by the method in Experiment 1 was prepared into a one µg/ml solution which was then distributed to the above microplate by 20-200 µl/well. To the microplate was further added a fresh preparation of the same buffer, supplemented with 500 µl/ml of Concanavalin A, by 10 µl/well, followed by the incubation at 37°C for 48 hours in a 5 v/v % CO₂ incubator. After completion of the culture, supernatants in each well were sampled by 0.1 ml/well, and determined for GM-CSF content using a conventional enzyme immunoassay. In parallel, a culture system free of IL-18 as a control was provided and treated similarly as above. The data is in Table 1:

Table 1

IL-18* (nM)	GM-CSF yield (pg/ml)
0	510
0.7	2,150

Table 1 (continued)

IL-18* (nM)	GM-CSF yield (pg/ml)
2.8	3,050
5.6	3,950
Note: The symbol *** means that IL-18 was a canavalin A.	dded to the culture system in the presence of 2.5 μg/ml of Con-

The results in Table 1 indicate that lymphocytes as an immunocompetent cell produced GM-CSF depending on the concentration of IL-18 when contacted with IL-18 in the presence of Concanavalin A as a cofactor. It was also confirmed that all of the IL-18 preparations and functional equivalents thereof, which were obtained by the methods in Experiments 2 to 5, induced GM-CSF production even when used alone similarly as above. An IL-18 preparation obtained by the method in Experiment 6 was tested in accordance with Experiment 7-1 except that the human lymphocytes used in the experiment were replaced with spleen cells prepared from mouse by a conventional manner, revealing that the IL-18 preparation also induced GM-CSF production.

Experiment 7-2

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Inhibition of osteoclast formation

Experiment 7-2(a)

As reported by T. J. Martin and K. W. Ng *in Journal* of Cellular *Biochemistry*, Vol. 56, pp. 357-366 (1994), it is considered requisite for contacting osteoclastic precursor cells, derived from hematopoietic stem cells, with osteoblasts or bone marrow stromas to generally differentiate osteoclastic precursor cells into mature osteoclasts. As described by G. D. Roodman in Endocrine *Reviews*, Vol. 17, No. 4, pp. 308-332 (1996), it is generally recognized that osteoclasts have characters of multinucleated cells, tartaric acid-resistant acid phosphatase (hereinafter abbreviated as "TRAP") activity, and a calcitonin receptor. In a co-culture system of osteoblasts and bone marrow cells as reported by Nobuyuki UDAGAWA et al., in *Journal of Experimental Medicine*, Vol. 182, pp. 1,461-1,468 (1995), these cells respond to factors such as 1α,25-dihydroxyvitamin D₃, prostaglandin E₂, adrenocortical hormone, interleukin 1, interleukin 6, and interleukin 11, to form osteoclast-like cells (hereinafter may be abbreviated as "OCL"). The formed OCL has characters of osteoclasts *in vivo*. Therefore, the co-culture system well reflects *in vitro* the processes of osteoclast formation in *vivo*. Using this system, experiments for osteoclast formation and osteoclastgenic inhibitory agents can be carried out.

The osteoclastgenic inhibitory activity of the IL-18 according to the present invention was studied using the above co-culture system. The osteoblasts used in this experiment were prepared in a conventional manner by treating a newborn mouse calvaria with 0.1 w/v % collagenase commercialized by Worthington Biochemical Co., Freefold, Australia, and 0.2 w/v % dispase commercialized by Godo Shusei Co., Ltd., Tokyo, Japan. The bone marrow cells were prepared from a mature mouse in a conventional manner. As a negative control, 2 x 104 cells of a primary cell culture of osteoblasts and 5 x 10⁵ cells of bone marrow cells were co-cultured in each well of a 48-well microplate containing 0.4 ml/well of α-MEM medium supplemented with 10 v/v % fetal calf serum (hereinafter designated as "Medium" throughout Experiment 4-2) at 37°C for seven days in a 5 v/v % CO₂ incubator. As a positive control, the above twotypes of cells were co-cultured similarly as in the negative control except that they were cultured in other wells containing 10⁻⁸M of 1α,25-dihydroxyvitamin D₃ commercialized by Wako Pure Chemicals, Tokyo, Japan, and 10⁻⁷M of prostaglandin E2 commercialized by Sigma Chemical Company, Missouri, USA. The aforesaid two-types of cells were cocultured similarly as in the positive control except that they were cultured in other wells containing 1a,25-dihydroxyvitamin D_3 commercialized by Wako Pure Chemicals, Tokyo, Japan, and prostaglandin E_2 commercialized by Sigma Chemical Company, Missouri, USA., in the same concentrations as used in the positive control, and a concentration of 0.01-10 ng/ml of an IL-18 preparation prepared by the method in Experiment 6. In every co-culture system, the media in each well were replaced with fresh preparations of the same media used in the co-culture systems on the 3rd day after the initiation of each culture. According to the method by Nobuyuki UDAGAWA in Journal of Experimental Medicine, Vol. 182, pp. 1,461-1,468 (1995), the cells on the 6th day after the initiation of each culture were fixed and stained based on TRAP activity, followed by counting the stained cells (hereinafter called "TRAP-positive cells") per well. Throughout Experiment 4-2, quadruplet wells under the same conditions were provided for each co-culture system, . and the mean value for the TRAP-positive cells per well in each system was calculated. The results are in Table 2:

Table 2

IL-18 (ng/ml)	Osteoclastgenic formation factor*1	Number of TRAP-positive cells per well"2	ells per well"2
0		2	
0	+	110	
0.01	+	114	
0.1	+	111	
0.5	+	106	
1	+	63	
2	+	29	
4	+	12	
8	+	2	
10	+	2	

The symbols of "+" and "-" show co-culture systems with and without $10^{-6}M$ Ia,25-dihydroxyvitamin D, and $10^{-7}M$ prostaglandin E, respectively. It shows a mean value of the data from quadruplet wells cultured under the same conditions. Note: 1:

*2:

As shown in Table 2, the formation of TRAP-positive cells was not substantially observed in the negative control, but the distinct formation was observed in the positive control. In the co-culture systems, i.e., the positive control supplemented additionally with IL-18, the formation of TRAP-positive cells was inhibited depending on the concentration of IL-18, and the maximum inhibition, i.e., a level equal to that in the negative control, was found at eight ng/ml or more of IL-18. These data strongly indicates that IL-18 has a concrete activity of inhibiting OCL formation in *vitro* and also inhibits osteoclast formation.

Experiment 7-2(b)

As described hereinbefore, it was confirmed that there exist factors that induce the formation of osteoclast-like cells in the co-culture systems used throughout Experiment 7-2. Therefore, in this Experiment 7-2(b), it was studied whether the inhibitory activity of IL-18 on osteoclast formation observed in Experiment 7-2(a) was specific to some factors or not; the osteoclast-like cells were cultured by the same method as used in the negative control in Experiment 7-2(a) except for using a medium supplemented with 10^{-8} M 1α ,25-dihydroxyvitamin D_3 , 10^{-7} M prostaglandin E_2 , 200 ng/ml parathyroid hormone, 100 ng/ml interleukin 1, or 20 ng/ml interleukin 11. These culture systems were for positive controls. In parallel, the cells were cultured in other wells by the same method used in the positive controls except for using a medium containing 10 ng/ml of an IL-18 preparation obtained by the method in Experiment 6, in addition to any one of the above factors at the same concentration. After completion of the cultures, TRAP-positive cells in each well were counted, and the numbers were compared similarly as in Experiment 7-2(a). The results are in Table 3:

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Table 3

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Osteoclast formation factor'l (concentration)	1 IL-18*2	Number of TRAP-positive cells per well*3
;	1	94
D3 (10 m)	+	8
	1	77
FGE ₂ (10 m)	+	3
	ı	63
Fin (200 ng/m1)	+	6
(10) (11)	1	84
(TIII / BII OOT) TT-T	+	3
11 (20 00)	r	71
(TIII/BII 07) T-T-	+	8

 D_3 , PGE₂, PTH, IL-11, and IL-1 are respectively $1\alpha,25$ -dihydroxyvitamin D_3 , prostaglandin E_2 , parathyroid hormone, interleukin-11, and interleukin-1 which were added to wells to give the concentrations as indicated in parentheses. The symbol "+" means that IL-18 was added to a well to give a concentration of 10 ng/ml, and the symbol "-" means that IL-18 was not added to. It shows a mean value of the data from quadruplet wells cultured under the same ;; *2: .. 3 Note:

conditions.



As shown in Table 3, a distinct formation of TRAP-positive cells was observed in every positive control, but the formation was almost completely inhibited in the presence of IL-18. This strongly indicates that IL-18 has a wide and general activity of inhibiting osteoclast formation independently of osteoclast-formation-related factors.

Experiment 7-2(c)

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It was studied whether the osteoclastgenic inhibition by IL-18, confirmed in Experiments 7-2(a) and 7-2(b), was caused by the action of the IL-18-induced GM-CSF. For positive and negative controls, the same co-culture systems employed in Experiment 7-2(a) were used. Using other wells, the co-culture of osteoblasts and bone marrow cells was carried out similarly as the method used for the positive controls except for using a medium supplemented with 1a, 25-dihydroxyvitamin D₃ and prostaglandin E₂ at the same concentrations used in the positive control, and with (i) 10 μg/ml of an anti-mouse GM-CSF polyclonal antibody commercialized by R&D Systems, Minnesota, USA, (ii) 10 ng/ml of an IL-18 preparation obtained by the method in Experiment 6, (iii) plus 10 µg/ml of an anti-mouse polyclonal antibody, (iv) 0.1 ng/ml of a mouse GM-CSF commercialized by R&D Systems, Minnesota, USA, or (v) (iv) plus 10 µg/ ml of an anti-mouse GM-CSF polyclonal antibody. After completion of the culture, TRAP-positive cells in each well were counted, and the numbers were compared similarly as in Experiment 7-2(a). The data is shown in Table 4 where the symbols "i" to "v" coincide with those used in the co-culture systems other than the control systems.

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GM-CSF Number of TRAP-positive ody*5 cells per well*6	8	122	112	Е	111	7	106
4 Anti-	ı	ı	+	,	+	1	+
GM-CSF	t	ı	,	,	ı	+	+
IL-18*3		t	1	+	+	ı	ı
Osteoclastgenic IL-18'3 GM-CSF'4 Anti-GM-CSF factor'2 antibody'5		+	+	+	+	+	+
Culture system'1	z	ል	1	11	111	iv	>

"1; where the symbols "N" and "P" mean negative and positive Note:

controls, respectively, and the symbols "i" to "v" correspond to those in the five types co-culture systems used.

where the symbol "+" means that 1a,25-dihydroxyvitamin D, and prostaglandin E, were respectively added to a well to give respective concentrations of 10°M and 10°M, and the symbol "-" means that these compounds were not added to.

The symbol "+" means that IL-18 was added to a well to give a concentration of 10 ng/ml, and the symbol "-" means that IL-18 2

was not added to. ښ ښ

The symbol "+" means that GM-CSF was added to a well to give a concentration of 0.1 ng/ml, and the symbol "-" means that GM-CSF 4;

was not added to. The symbol "+" means that an anti-GM-CSF polyclonal antibody was added to a well to give a concentration of $10~\mu g/ml$, and the symbol "-" means that the polyclonal antibody was not added to. . 5

As shown in Table 4, the formation of TRAP-positive cells was almost completely inhibited by IL-18, cf., the co-culture system (ii), but the inhibition was almost completely inhibited by the addition of the anti-mouse polyclonal antibody, cf., the co-culture system (iii). Mouse GM-CSF exhibited an activity of inhibiting the formation of TRAP-positive cells similar to IL-18, cf., the co-culture system (iv), and the inhibition was almost completely inhibited by the addition of the anti-mouse GM-CSF polyclonal antibody, cf., the co-culture system (v). The sole use of the anti-mouse GM-CSF polyclonal antibody gave no influence on the formation of TRAP-positive cells, cf., the co-culture system (i). These data strongly indicates that the osteoclastgenic inhibition by IL-18 was due to the action of the IL-18-induced GM-CSF.

Experiment 8

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Acute toxicity test

Eight-week-old mice were in a conventional manner injected percutaneously, orally, or intraperitoneally with either of IL-18 preparations obtained by the methods in Experiments 1 to 6. The results showed that these IL-18 preparations had an LD₅₀ of about one mg/kg or more in mice independent of the route of administration. The data evidences that IL-18 can be incorporated into pharmaceuticals for warm-blooded animals in general and including humans without causing no serious side effects.

As described in *Nikkei Biotechnology Annual Report 1996*, pp. 498-499 (1995), published by Nikkei BP Publisher, Tokyo, Japan (1995), the IL-18-induced GM-CSF has not yet been clinically used in Japan, but applied clinically in USA and Europe. The fact would show that IL-18 has substantially no serious side effects. These facts indicate that the osteoclastgenic inhibitory agent according to the present invention can be successively administered to warm-blooded animals in general and including humans to induce osteoclast formation and exert a satisfactory therapeutic and/or prophylactic effect on osteoclast-related diseases without causing serious side effects.

The following Examples describe the present osteoclastgenic inhibitory agent according to the present invention:

Example 1

<u>Liquid</u>

Either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in physiological saline containing one w/v % human serum albumin as a stabilizer to give a concentration of two mg/ml of the IL-18 preparation. The resulting solutions were in a conventional manner membrane filtered for sterilization into liquids.

The liquids have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of an injection, ophthalmic solution, or collunarium for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 2

Dry agent

Fifty milligrams of either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in 100 ml of physiological saline containing one w/v % purified gelatin as a stabilizer. The solutions thus obtained were in a conventional manner membrane filtered for sterilization, distributed to vials by one milliliter, lyophilized, and sealed with caps.

The products have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of a dry injection for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 3

Dry agent

Fifty milligrams of either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in 100 ml of physiological saline containing one w/v % trehalose as a stabilizer. The solutions were in a conventional manner membrane filtered for sterilization, distributed to vials by one milliliter, lyophilized, and sealed with caps.

The products have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of a dry injection for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 4

Ointment

HIVIS WAKO GEL® 104, a carboxyvinylpolymer commercialized by Wako Pure Chemical Industries, Ltd., Tokyo, Japan, and a high-purity trehalose were dissolved in a sterilized distilled water to give respective concentrations of 1.4 w/w % and 2.0 w/w %, and the solution was mixed to homogeneity with either of IL-18 preparations obtained by the methods in Experiments 1 to 6, and adjusted to pH 7.2 to obtain a paste containing about one mg of an IL-18 preparation per g of the product.

Each product thus obtained has a satisfactory spreadability and stability and can be arbitrarily used as an agent in the form of an ointment for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 5

Tablet

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"FINETOSE®", an anhydrous crystalline α -maltose powder commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, was mixed to homogeneity with either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, and "LUMIN" or 1-1'-1"-trihepthyl-11-chinolyl(4)*4*-penthamethinchynocyanine-1,1"-dijodide. The mixtures were in a conventional manner tabletted to obtain tablets, about 200 mg weight each, containing an about two milligrams of either of the IL-18 preparations and an about two milligrams of LUMIN per tablet.

The products have a satisfactory swallowability, stability, and cell-activating activity and can be arbitrarily used as agents in the form of a tablet for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

As described above, the osteoclastgenic inhibitory agent according to the present invention effectively inhibits osteoclast formation. Therefore, the agent can be arbitrarily used as an ingredient for cell culture and agents for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Thus the present invention with these useful activities and functions is a significant invention that would greatly contribute to this field.

While there has been described what is at present considered to be the preferred embodiments of the invention, it will be understood the various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirits and scope of the invention.

Annex to the description

5			SEQ	JENCE LI	STING	
	(1)	INFORMATION	FOR SEQ I	D NO: 1:	:	
10		(B)TYP	CHARACTER GTH: 6 ami E: amino a DLOGY: lin	no acids cid	;	
		(11)MOLECUL	E TYPE: pe	ptide		
15		(v)FRAGMENT	TYPE: int	ernal fr	agment	
		(xi)SEQUENC	E DESCRIPT	ION: SEC	ID NO:	1:
20	Asn A	Asp Gln Val :	Leu Phe			
25	(2)	(B)TYP	_	ISTICS: no acids cid		
		(ii)MOLECUL	E TYPE: in	ternal f	fragment	
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50	(i	(B)TYPE:	ARACTERIST : 5 amino amino acid GY: linear	acids		
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	Ser A	sp Ile	Ile P	he Phe	Gln	Arg	Ser 105	Val	Pro	Gly	His	Asp 110	Asn	Lys
45	Met G	ln Phe 115		er Ser	Ser	Tyr 120		Gly	Tyr	Phe	Leu 125		Cys	Glu
		lu Arg	Asp L	eu Phe	Lys 135		Ile	Leu	Lys	Lys 140	_	Asp	Glu	Leu
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	65	Lys	_			70					75	_		_		80	
20		Phe			85	_				90		_	_		95		
20	Λ^{-}	Leu		100			_	_	105		_			110			
		Glu	115			_		120					125		-		
25	_	Asp 130					135					140		Asn	Gly	Asp	
	Lys 145	Ser	Val	Met	Phe	Thr 150	Leu	Thr	Asn	Leu	His 155	Gln	Ser				
	(8)	INFO	RMAT	ON I	FOR S	SEQ 1	D NO): 8:	:								
30 35		į)	(A)LEN B)TYI C)STI	E CHA GTH: PE: 1 RANDE POLOG	471 ucle	l bas eic a SS: c	se pa acid loub]	airs						-		
33		(i:	L)MOI	LECUI	LE TY	PE:	CDNA	A									
40		(v:	(P	()ORG	AL SC SANIS	sm: h	umar										
40		(1:	(E	NAN B)LOC	E/KE)N: 1	447	71	lde iod:	E							
45		(ж	L)SEQ	UENC	E DE	SCRI	PTIC	ON: S	SEQ 1	D NC	: 8:						
	TAC TT																48
50	GAC CA			5			_		10					15			96
	Asp G	.n Va	1 Le	u Ph	e Il	e As	p Gl	n Gl 25		n Ar	g Pr) Le	u Pho 30	e Glu	a Ası	þ	
	ATG AC	T GA	T TC	T GA	C TG	T AG	A GA	T AA	T GC.	A CC	C CG	G AC	C AT	A TTI	AT:	r	144

	Met	Thr	Asp 35	Ser	Asp	Căa	Arg	Asp 40	Asn	Ala	Pro	Arg	Thr 45	Ile	Phe	Ile	
	ATA	AGT		TAT	AAA	GAT	AGC		CCT	AGA	GGT	ATG	GCT	СТА	аст	a Tro	192
5													Ala				172
	TCT		AAG	TGT	GAG	AAA		TCA	ACT	CTC	TCC		GAG	AAC	AAA	ATT	240
	Ser 65	Val	Lys	Cys	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile 80	240
10	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA		288
10	Ile	Ser	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	Lys	200
	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT		AAG	336
	Ser	Asp	Ile	Ile 100	Phe	Phe	Gln	Arg	Ser 105	Val	Pro	Gly	His	Asp 110	Asn	Lys	-
15	ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
			115					120					Leu 125				
	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
20		130					135					140		Asp	Glu	Leu	
					ATA												471
	145	Asp	Arg	ser	Ile	150	Pne	Thr	VaI	GIn	155	Glu	Asp				
25	(9)	IN	FORM	ATIO	V FOE	R SEC) ID	NO:	9:								
		(1)	() (I	A)LEI B)TYI	E CHANGTH: PE: 8	: 11 mino	amir aci	no ac ld									
30		(i:	L)MOI	LECUI	LE TY	PE:	pept	ide									
		(v)	FRAC	SMENT	r TY	E: N	I-tei	mina	ıl fı	agme	ent					-	
35		(x)	i)seç	QUENC	CE DE	ESCRI	PTIC	on: s	EQ I	D NC): 9:						
	Met 1	Tyr	Phe	Gly	Lys 5	Leu	Glu	Ser	Lys	Leu 10	Ser						
	(10)	INE	ORM	ATION	FOF	SEC) ID	NO:	10:								
40		(1)	(<i>F</i>	A)LEN	CHA GTH: PE: a POLOG	10 mino	amin aci	os or .d									
45		(11	.)MOI	LECUI	E TY	PE:	pept	ide									
		(v)	FRAC	MENI	TYP	E: C	-ter	mina	l fr	agme	nt						
50		(xi)SEC	UENC	E DE	SCRI	PTIC	N: S	EQ I	D NO	: 10	:					
	Ser 1	Ile	Met	Phe	Thr 5	Val	Gln	Asn	Glu	Asp 10							

	(11) INFORMATION FOR SEQ ID NO: 11:
5	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 13 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear
10	(ii)MOLECULE TYPE: peptide
,,,	(v)FRAGMENT TYPE: N-terminal fragment
	(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 11:
15	Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg 1 10
	(12) INFORMATION FOR SEQ ID NO: 12:
20	<pre>(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 14 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear</pre>
	(i1)MOLECULE TYPE: peptide
25	(v)FRAGMENT TYPE: internal fragment
	(x1)SEQUENCE DESCRIPTION: SEQ ID NO: 12:
30	Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg 1 10
	(13) INFORMATION FOR SEQ ID NO: 13:
35	<pre>(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 17 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear</pre>
	(ii)MOLECULE TYPE: peptide
40	(v)FRAGMENT TYPE: internal fragment
	(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 13:
45	Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 1 10 15
	(14)INFORMATION FOR SEQ ID NO: 14:
50	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 471 base pairs (B)TYPE: nucleic acid (C)STRANDEDNESS: double (D)TOPOLOGY: linear

		(ii)MOL	ECUL	E TY	PE:	CDNA	•									
5		(ix	(B)NAM	E/KE ATIO	Y: m N: 1	47	1		s							
10		(xi)SEQ	UENC	E DE	SCRI	PTIO	N: S	EQ I	D NO	: 14	:					
10	ma.c	መውጥ	ccc	AAG	СФФ	GAA	Tr CT	222	ጥጥል	ሞሮል	GTC	ΔΤΔ	AGA	таа	ጥጥር	ΔΔΤ	48
	Tyr 1	Phe	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10	Val	Ile	Arg	Asn	Leu 15	Asn	40
15	GAC Asp	CAA Gln	GTT Val	Leu	TTC Phe	ATT Ile	GAC Asp	CAA Gln	Gly	AAT Asn	CGG Arg	CCT Pro	CTA Leu	Phe	GAA Glu	GAT Asp	96
	ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	25 AAT Asn	GCA Ala	CCC	CGG	ACC Thr	30 ATA	TTT Phe	ATT	144
	Mec	1111	35	Ser	ngp	Jei	AL Y	40	11311			9	45				
20	ATA Ile	Ser	ATG Met	TAT Tyr	AAA Lys	GAT Asp	Ser	CAG Gln	CCT Pro	AGA Arg	GGT Gly	ATG Met 60	GCT Ala	GTA Val	ACT Thr	ATC Ile	192
	ጥርጥ	50 СТС	AAG	тст	GAG	AAA	.55 ATT	TCA	ACT	CTC	TCC		GAG	AAC	AAA	АТТ	240
	Ser 65	Val	Lys	Ser	Glu	Lys 70	Ile	Ser	Thr	Leu	Ser 75	Ala	Glu	Asn	Lys	Ile 80	
25					Glu					Asp			AAG Lys		Thr		288
	» Cm	CNC	እጥሮ	ስ T' ስ	85 ****C	ттт	CNG	AGA	ACT	90 GTC	CCA	GGA	CAT	САТ	95 AAT	AAG	336
	Ser	Asp	Ile	Ile 100	Phe	Phe	Gln	Arg	Ser 105	Val	Pro	Gly	His	Asp 110	Asn	Lys	000
30				GAA									CTA				384
			115					120					Leu 125			-	400
	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA Lvs	AAA Lve	GAG Glu	GAT	GAA	TTG	432
35	гåг	130	ALG	nsp	Dea	1116	135	Deu	116	DCu	_, _	140	0				
,,,		GAT															471
	Gly 145	Asp	Arg	Ser	IIe	Met 150	Pne	Thr	vai	GIN	155	GIU	Asp				
40	(15) IN	FORM	ATIO	v FOI	R SE	O ID	NO:	15:								
		(i	(1	A)LEI B)TYI	NGTH:	ARAC : 10 amin GY: :	ami ac.	no ao id									
45 -		(i:	i)MO	LECU	LE T	YPE:	pep	tiđe									
		(v)FRA	GMEN'	r TY	PE: 1	N-te:	rmina	al f	ragmo	ent						
50		(x:	i)SE	QUEN	CE DI	ESCR:	IPTI:	on:	SEQ	ID N): 1	5:					
<i>3</i> 0	Tyr	Phe	Gly		Leu 5			Lys									

	(10	LINE		1 1014	FOR	SEQ	10	NO	LU.								
5		(1	(1	A)LEI B)TYI C)STI	NGTH PE: 1 RAND	ARACT : 47: nucle EDNES GY:	l bas	se pa acid doub	airs								
		(i.	i)MO	LECU	LE T	YPE:	CDN	A.									
10					_												
		(1:	ÇI	A)NAI	ME/KI	EY: I ON: : FICA:	L4	71		s							
15		(x :	i)SE(QUENC	CE DI	ESCR	[PTI	on: s	SEQ :	ID N	0: 1	5 :					
	TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
	. -	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	_	Val	Ile	Arg	Asn		Asn	
20	l CAC	CAA	CTT	CTC	5 mmc	3.00	CNC	CNA	CCN	10	ccc	CCM	Cm x	mmm	15	C 3 M	0.0
20		CAA Gln															96
	ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT		GCA	CCC	CGG	ACC		TTT	ATT	144
	Met	Thr		Ser	Asp	Ser	Arg	-	Asn	Ala	Pro	Arg		Ile	Phe	Ile	
25	a m a	AGT	35	TO A ITT		CATT	ACC.	40	CCB	n C'n	ccm	a mo	45 CC	Cm a	3 CM	3 mc	100
		Ser															192
		50		-1-	-1-		55			9	1	60					
		GTG															240
30	Ser 65	Val	Lys	Ser	GIu	Lys 70	IIe	Ser	Thr	Leu	Ser 75	Ala	GLu	Asn	Lys	Ile 80	
30		TCC	TTT	AAG	GAA		AAT	CCT	CCT	GAT		ATC	AAG	GAT	ACA		288
		Ser															
		GAC															336
35		Asp		100				_	105			_		110		_	224
		CAA Gln							_	_				_			384
		U	115	0_0				120		017	-1-		125			014	
		GAG															432
40	_	Glu 130	_	_			135					140		Asp	Glu	Leu	
		GAT Asp															471
	145	vab	ALG	Ser	116	150	FILE	1111	491	GIII	155	Gid	лэр				
45	(17))INF	ORMAT	rion	FOR	SEQ	ID !	10: 1	l 7:								
50		(1)	(E	A)LEN B)TYF C)STF	IGTH: PE: r RANDE	RACT 114 ucle DNES	64 bic 6	oase acid loubl	pain	:s							
			, .	,, 101	J-00												

	(ii)MOLECULE TYPE: genomic DNA	
	(vi)ORIGINAL SOURCE:	
_	(A)ORGANISM: human	
5	(G)CELL TYPE: placenta	
	(c,c)	
	(ix)FEATURE:	
	(A)NAME/KEY: 5 UTR	
	(B)LOCATION: 13	
10	(C)IDENTIFICATION METHOD: E	
	(A)NAME/KEY: leader peptide	
	(B)LOCATION: 482	
	(C)IDENTIFICATION METHOD: S	
	(A)NAME/KEY: intron	
15	(B)LOCATION: 831453	
	(C)IDENTIFICATION METHOD: E	
	(A)NAME/KEY: leader peptide	
	(B)LOCATION: 14541465	
	(C)IDENTIFICATION METHOD: S	
	(A)NAME/KEY: intron	
20	(B)LOCATION: 14664848	
	(C)IDENTIFICATION METHOD: E	
	(A)NAME/KEY: leader peptide	
	(B)LOCATION: 48494865	
	(C)IDENTIFICATION METHOD: S	
25	(A)NAME/KEY: mat peptide	
	(B)LOCATION: 48664983	
	(C)IDENTIFICATION METHOD: S	
	(A)NAME/KEY: intron (B)LOCATION: 49846317	
	(C)IDENTIFICATION METHOD: E	
30	(A)NAME/KEY: mat peptide	
	(B)LOCATION: 63186451	
	(C)IDENTIFICATION METHOD: S	
	(A)NAME/KEY: intron	
	(B)LOCATION: 645211224	
25	(C)IDENTIFICATION METHOD: E	
35	(A)NAME/KEY: mat peptide	
	(B)LOCATION: 1122511443	
	(C)IDENTIFICATION METHOD: S	
	(A)NAME/KEY: 3´ UTR	
	(B)LOCATION: 1144411464	
40	(C)IDENTIFICATION METHOD: E	
	(x1)SEQUENCE DESCRIPTION: SEQ ID NO: 17:	
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	Met Lys Phe Ile Asp Asn Thr Leu Tyr Phe Ile Ala	,,
	-20 -15 -10	
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50	ATTAAGTGAC TCTTTGTGTC ACCAAATTTC ACTGTAATAT TAATGGCTCT TAAAAAAATA	218
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	GTCCTGGCAC TTTAATCAGC AGTAGCTCAC TCTCCAGTTG GCAGTAAGTG CACATCATGA	338

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                                                                                  818
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                                                                                  998
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15
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       1890
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                                                                                 1950
                                                                                 2010
                                                                                 2070
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15	ACAAATAAGT TAGGGATTTA ATATCCTGGC CAAATGGTAG ACAAAATGAA CTCTGAGATC	4470
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20	CTAAAATATA TAGCATACTT ATTTGTCAAT TAACAAAGAA ACTATGTATT TTTAAATGAG	4830
	ATTTAATGTT TATTGTAG AA AAC CTG GAA TCA GAT TAC TTT GGC AAG CTT	4880
	Glu Asn Leu Glu Ser Asp Tyr Phe Gly Lys Leu	
	-5 1 5	
	GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT GAC CAA GTT CTC TTC	4928
25	Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe	
	10 15 20	
	ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT ATG ACT GAT TCT GAC	4976
	Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp	
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	CTGTAGTACA CTGTGATCGT ACCTGTGAAT AGCCACTGCA CTCCAGCCTG GGTGATATAC	5452
	AGACCTTGTC TCTAAAATTA AAAAAAAAAA AAAAAAAAAC CTTAGGAAAG GAAATTGATC	5512
	AAGTCTACTG TGCCTTCCAA AACATGAATT CCAAATATCA AAGTTAGGCT GAGTTGAAGC	5572
40	AGTGAATGTG CATTCTTTAA AAATACTGAA TACTTACCTT AACATATATT TTAAATATTT	5632
	TATTTAGCAT TTAAAAGTTA AAAACAATCT TTTAGAATTC ATATCTTTAA AATACTCAAA	5692
	AAAGTTGCAG CGTGTGTGTT GTAATACACA TTAAACTGTG GGGTTGTTTG TTTGTTTGAG	5752
	ATGCAGTTTC ACTCTGTCAC CCAGGCTGAA GTGCAGTGCA	5812
	CTCACTACAA CCTCCACCTC CCACGTTCAA GCGATTCTCA TGCCTCAGTC TCCCGAGTAG	5872
	GTGGGATTAC AGGCATGCAC CACTTACACC CGGCTAATTT TTGTATTTTT AGTAGAGCTG	5932
45	GGGTTTCACC ATGTTGGCCA GGCTGGTCTC AAACCCCTAA CCTCAAGTGA TCTGCCTGCC	5992
	TCAGCCTCCC AAACAAACAA ACAACCCCAC AGTTTAATAT GTGTTACAAC ACACATGCTG	6052
	CAACTTTTAT GAGTATTTTA ATGATATAGA TTATAAAAGG TTGTTTTTAA CTTTTAAATG	6112
	CTGGGATTAC AGGCATGAGC CACTGTGCCA GGCCTGAACT GTGTTTTTAA AAATGTCTGA	6172
	CCAGCTGTAC ATAGTCTCCT GCAGACTGGC CAAGTCTCAA AGTGGGAACA GGTGTATTAA	6232
50	GGACTATCCT TTGGTTAAAT TTCCGCAAAT GTTCCTGTGC AAGAATTCTT CTAACTAGAG	6292
	TTCTCATTTA TTATATTTAT TTCAG AT AAT GCA CCC CGG ACC ATA TTT ATT	6343
	Asp Asn Ala Pro Arg Thr Ile Phe Ile	

				40		45	
	ATA AGT	ATG TAT	AAA GA'		CCT AGA GGT	ATG GCT GTA ACT ATC	6391
						y Met Ala Val Thr Ile	
5	50	,-	-10	55		60	
		AAC TOT	GAG AA		ACT CTC TCC	TGT GAG AAC AAA ATT	6439
						r Cys Glu Asn Lys Ile	0.03
	65	nia cia	70		75		
		מאם ייייי				CAATCATGTT AATATAATCA	6496
	Ile Ser			.0100001			. 0270
10				የጥልጥጥጥር ጥል	י מדמד מי מי מי	A AGTAATGTAA TTAGAAAACI	6556
						A ACAAGAAGCA GAGAACCATI	
						TTGTGATAAT GATGGTTTTT	
						TTATGACCTG CATCTCCTGA	
						r gtgagttata catttaagaa	
4.5						C GAAGCTAATT ATCCTTCTAT	
15						TAGTTGTTTT GTTGCTGATO	
						G ATGTATGTTA TTTTTAATGT	
						G TAATGCTATA ATTATCTTCA	
						A TTATTCTCCA TTATTATTCT	
						A CCACAATTAA CTATAGCTAC	
20						r ggcaatgctt cagaggagaa	
						A ATAAATATCC GCTTTCATGO	
						r agtgaaggta ccaaggggca	
						A AGTAAGAACA GTGCATATGC C TCCAACCAGA GTGCCACCCC	
						G GCAGCTTAGT TATCAAAATA	
25							
						r taagcatget gttaetgaac A tgtgggatag aggaaaacto	
						CTACAGGTGG ATTCTTGTTG	
						TTGGCACTTA GTAGGAACTG	
						TIGGCACITA GIAGGAACIG	
30						CAGCACTTTG GGAGGCCGAG	
30						GACCAACATG GAGAAACCCC	
						A TATGCCTGTA ATCCCAGCTA	
						G GCAGAGGTTG CGATGAGCCT A AACTCGGTCT CAAAAAAAAA	
35						A TTTAATACTG TTTTTAAGTA	
						A ATCTGACATT TAAGCTTCAT	
						A ATATTAGTTG GAGGGGGGGA	
						TTTAATCCCT TTTCCTGCCA	
						TTCTAGATAA TAAGATACAA	
						TTGGGAGGC AAGGCGAGTG	
40						ATGGCGATAC TCTGTCTCTA	
	CTAAAAAA	AA TACA	AAAATT A	AGCCAGGCA	r GGTGGCATG	ACCTGTAATC CCAGCTACTC	8656
						TAGGCTGCAG TGAGCTGAGA	
						TTGTCTCAAA AAAAGAAAAA	
						GATTACTAGC TATAAAGTCC	
45						GGATTTGCTT TGAGAGGTTA	
70						A TATGTATCTA TATCCAGGCT	
						TGTTAGGTCA ATATCAACTT	
	TCCCTGGA	TT CAGA	TICAAC (CCTTCTGA'	r Graaaaaaa	A AAAAAAAAA GAAAGAAATC	9016
	CCTTTCCC	CT TGGA	GCACTC /	AGTTTCAC	AGGTGGGGC	TTCCAAGTTG GGGGTTCTCC	9076
	AAGGTCAT	TG GGAT	TGCTTT (ACATCCAT	r rectatetac	CTTCCCTATG ATGGCTGGGA	9136
50						GTCCTTACCT CTATTCTGAA	
						TATCCACACT CTCGCTTTCA	
	ACTGTAAC	TT TCTT	TTTTTC :	"TTTTTTCT"	r tttttcttt	TTTTTGAAAC GGAGTCTCGC	9316

	TCT	STCG	CCC	AGGC	TAGAC	T G	CAGT	GGCAC	GA'	CTC	AGCT	CAC	rgca.	AGC	TCTC	CCTCCC	9376
																CTGCCA	9436
											– –					AGCCAGG	9496
5																r GGGATT	9556
																CTTCCCC	9616
														-		CATTAG	9676
																STATATT	9736
																AACTTAG	9796
																CAACTG	9856
10																ATCTTTG	9916
																ATCATGG	9976
																GATTAA	10036
						-	_		_			_		-		CATGCT	10096
												_				TAGTTT	10156
15																BAATCTG	10216
13						_	_			_	-					TTCAAG	10216
																TGAGCC	10336
											_					TCTATT	10336
																CACTTTC	10396
											-	-				CTTACA	10436
20																AGTTCA	10516
																'AAATTT	10636
		-														CTGAGG	10696
													_			CACTGC	10756
																ACTAGA	10816
																TTATAG	10876
25																TTAATC	10936
				-	-											GTATAG	10996
									-		-					GATTCG	11056
																CTCTTG	11116
																TATATTC	11176
30	-															G AAT	11233
	AAA.	I I G I .	LCH	1010	CIGM	V	W111	10017		<i>3</i> 111.		CIC.				et Asn	11255
							•	•						8		et gan	
	ССТ	ССТ	САТ		ATC	244	CAT	ACA.	444	ACT	GAC	ΔТС	ата			CAG	11281
			-	-		_					_	_				e Glu	11201
	FIO	FIO	90	, voii	***	Dy 3	rsp.	95	ny 3	Ser	LSP	116	100		- I II	e Giu	
35	AGA	ACT		CCA	CCA	САТ	САТ		DAG	ΔТС	440	ጥጥጥ			ጥር ል	TCA	11329
																r Ser	11327
	ALG	105	V G I	FIO	GLY	птэ	110		Dys	1-16-0	Gin	115		96.		r ser	
	TAC		CCA	ጥልሮ	ጥጥጥ	СТА			CAA	444	GAG			Стт	do chodo	' AAA	11377
																e Lys	113//
40	120	GIU	GLY	- Y	* * * * * * * * * * * * * * * * * * * *	125	AIG	Cys	G1 4	. Lys	130	arg	ngp	ne.		135	
40		חיים ע	ተነጥር	888	444		CAT	CAA	ጥጥር	GGG		AGA	ጥርጥ	АТА	ልሞር	TTC	11425
																t Phe	11423
	Tea	116	Ten	nys.	140	JIU	vəħ	Jiu	neu	145	vaħ	ur A	261		150		
	A CTT	Crim	CAN	220	GAA	CAC	TAC	ייי בייי	י גמי		ייים	בר ר			130	_	11464
		-			Glu		TUG	TALL		. I 170	·~~1	3 0 0					77404
45	¥ 114	AGT	GIII	155	31 .u	vaħ											
				133													

(18) INFORMATION FOR SEQ ID NO: 18:

(i)SEQUENCE CHARACTERISTICS:
 (A)LENGTH: 471 base pairs
 (B)TYPE: nucleic acid
 (C)STRANDEDNESS: double

. 55

			(1	D)TO	POLO	GY:	linea	ar									
5		(1:	L)MO	LECU	LE T	YPE:	CDN	A to	mRN.	A							
J		(v:	i)OR:		AL S GANI			9									
			((G)CE	LL T	YPE:	live	er									
10		(i.		IAN (A	ME/KI				ide								
					ENTI				HOD:	s							
		(x:	L)SE(QUEN	CE DI	ESCR:	IPTIC	эи: :	SEQ :	ID N): 18	В:					
15	AAC	TTT	GGC	CGA	CTT	CAC	TGT	ACA	ACC	GCA	GTA	ATA	CGG	AAT	ATA	AAT	48
		Phe															
		CAA															96
20	-	Gln		20			_		25					30	-		
		GAT Asp															144
	1112	,,op	35		02			40					45				
		ATG															192
25	_	Met 50	_	_	_		55		_	_		60					
		AAG Lys															240
	65	TTT	_		_	70					75					80	288
30		Phe															
		CTC															336
		Leu		100					105					110			
35		GAA										_					384
		Glu	115			-		120					125		_		432
		GAT Asp															432
		130			-2-		135		-1-	-1-	-4 -	140			2		
40		TCT															471
	Lys 145	Ser	Val	Met	Phe	Thr 150	Leu	Thr	Asn	Leu	His 155	Gln	Ser				
	(19) INI	FORM	TION	V FOI	R SEC] ID	NO:	19:								
45		(±)	(E	\)LEI	E CHA NGTH: PE: 6 POLOG	9 a	amino aci	aci ld									
50		(ii	L)MOI	LECUI	LE TY	PE:	pept	tide									
		(v)	FRAC	MENT	TYI	PE: N	l-te:	cmina	al fi	agme	ent						

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 19: Asn Phe Gly Arg Leu His Cys Thr Thr 5 (20) INFORMATION FOR SEQ ID NO: 20: (i) SEQUENCE CHARACTERISTICS: 10 (A) LENGTH: 157 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20: Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 20 20 25 30 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 45 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 60 25 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 70 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 85 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 30 100 105 110 Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 115 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 130 135 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 35 150 (21) INFORMATION FOR SEQ ID NO: 21: (1) SEQUENCE CHARACTERISTICS: 40 (A) LENGTH: 157 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21: Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 50

25 Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile

45

40

Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile

20

35

Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val. Gln Asn Glu Asp

(22) INFORMATION FOR SEQ ID NO: 22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Ly<u>s</u> Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp

(23) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 7Ō Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp

(24) INFORMATION FOR SEQ ID NO: 24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
- (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ser Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp

- (25) INFORMATION FOR SEQ ID NO: 25:
 - (i) SEQUENCE CHARACTERISTICS:(A) LENGTH: 157 amino acids

- (B) TYPE: amino acid
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:
- Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile 0 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 9Ō Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
 - (26) INFORMATION FOR SEQ ID NO: 26:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:
- Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 115 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu

5	Gly 145	130 Asp	Arg	Ser	Ile	Met 150	135 Phe	Thr	Val	Gln	Asn 155	140 Glu	Asp			
	(27) INI	FORM	ATIO	v FOI	R SE	Q ID	NO:	27:			٠				
10		(:	(E	EQUENA) LI B) TY C) T(ENGT! YPE:	H: 19 amin	57 ar	mino cid		ds		•				
		(:	ii) N	MOLEC	CULE	TYPI	E: pe	eptio	ie.							
15		(:	кі) S	SEQUE	ENCE	DES	CRIP	rion	: SE(Q ID	NO:	27:				
	Asn 1	Phe	Gly	Arg	Leu 5	His	Ala	Thr	Thr	Ala 10	Val	Ile	Arg	Asn	Ile 15	Asn
20	Asp	Gln	Val	Leu 20	Phe	Val	Asp	Lys	Arg 25	Gln	Pro	Val	Phe	Glu 30	Asp	Met
	Thr	Asp	Ile 35	Asp	Gln	Ser	Ala	Ser 40	Glu	Pro	Gln	Thr	Arg 45	Leu	Ile	Ile
	Tyr	Met 50	Tyr	Lys	yab	Ser	Glu 55	Val	Arg	Gly	Leu	Ala 60	Val	Thr	Leu	Ser
25	Val 65	Lys	Asp	Ser	Lys	Met 70	Ser	Thr	Leu	Ser	Cys 75	Lys	Asn	Lys	Ile	Ile 80
	Ser	Phe	Glu	Glu	Met 85	Asp	Pro	Pro	Glu	Asn 90	Ile	Asp	Asp	Ile	Gln 95	Ser
30	_		Ile	100			-	_	105					110		
			Ser 115					120					125			
	_	130	Ala				135		_	_		140		Asn	Gly	Asp
35	Lys 145	Ser	Val	Met	Phe	150	Leu	Thr	Asn	Leu	H1S 155	Gln	Ser			
	(28) INI	FORMA	OITA	1 FOI	R SEC) ID	NO:	28:							
40		()	(E	EQUEN A) LE B) TY O) TO	ENGTI PE:	f: 15	7 an	onino bic		is						
45		()	(1) N	OLEC	CULE	TYPE	E: pe	ptic	le							
		()	ki) S	SEQUE	ENCE	DESC	CRIPT	: NOI	SEC] ID	NO:	28:				
	Asn 1	Phe	Gly	Arg	Leu 5	His	Cys	Thr	Thr	Ala 10	Val	Ile	Arg	Asn	Ile 15	Asn
50		Gln	Val	Leu	Phe	Val	Asp	Lys	Arg		Pro	Val	Phe	Glu		Met

Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile 35

Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser 50

Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile

65 Ser	Phe	Glu	Glu	Met 85	70 Asp	Pro	Pro	Glu	Asn 90	75 Ile	Asp	Asp	Ile	Gln 95	80 Ser
Ąsp	Leu	Ile	Phe 100	Phe	Gln	Lys	Arg	Val. 105	Pro	Gly	His	Asn	Lys 110	Met	Glu
Phe	Glu	Ser 115	Ser	Leu	Tyr	Glu	Gly 120	His	Phe	Leu	Ala	Ser 125	Gln	Lys	Glu
Asp	Asp 130	Ala	Phe	Lys	Leu	Ile 135	Leu	Lys	Lys	Lys	Asp 140	Glu	Asn	Gly	Asp
Lys 145	Ser	Val	Met	Phe	Thr 150	Leu	Thr	Asn	Leu	His 155	Gln	Ser			

SEQUENCE LISTING

	(1) GENERAL INFORMATION:
5	(i) APPLICANT: NAME: KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO
	(ii) TITLE OF INVENTION: OSTEOCLASTGENIC INHIBITORY AGENT
10	(iii) NUMBER OF SEQUENCES: 28
15	(iv) ADDRESS: (A) ADDRESSEE: KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU KENKYUJO (B) STREET:2-3, 1-CHOME, SHIMOISHII (C) CITY: OKAYAMA (E) COUNTRY: JAPAN (F) POSTAL CODE (ZIP): 700
20	(v) COMPUTER READABLE FORM:(A) MEDIUM TYPE: Floppy disk(B) COMPUTER: IBM PC compatible(C) OPERATING SYSTEM: PC-DOS/MS-DOS
25	(vii) PRIOR APPLICATION DATA: (A1) APPLICATION NUMBER: JP 55,468/1997 (B1) FILING DATE: 25-FEB-1997
	(2) INFORMATION FOR SEQ ID NO: 1:
30	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 6 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (v) FRAGMENT TYPE: internal fragment (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:
35	Asn Asp Gln Val Leu Phe 1 5
	(3) INFORMATION FOR SEQ ID NO: 2:
40	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 6 amino acids (B)TYPE: amino acid (D)TOPOLOGY: linear
45	(ii) MOLECULE TYPE: internal fragment
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:
	Phe Glu Asp Met Thr Asp 1 5
50	(4) INFORMATION FOR SEQ ID NO: 3:
55	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 7 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear

		(i:	L) MOI	LECUI	LE T	YPE:	pep	tide								
		(v)	FRAC	MEN.	r TY	PE:	inte	rnal	fra	gmen	t					
5		(x:	L) SE(QUEN	CE DI	ESCR:	IPTI	ON:	SEQ :	ID N	D: 3	:				
	Phe 1	Lys	Leu	Ile	Leu 5	Lys	Lys									
10	(5)	INI	FORM	ATIO	N FOI	R SE	Q ID	NO:	4:							
15		(i)	(1	4) LEI 3) TYI	NGTH PE: a	ARAC : 5 a amin GY: .	amino o ac:	o ac: id								
		(i:	L) MOI	LECUI	LE T	YPE:	int	erna	l fra	agmei	nt					
		(x:	i)se(QUEN	CE DI	ESCR:	IPTI	ON:	SEQ :	ID N): 4	:				
20	Met 1	Tyr	Lys	Asp	Ser 5											
	(6)	IN	FORM	ATIO	I FOI	R SE	Q ID	NO:	5:							
25		(i)	(1	A) LEI 3) TYI	NGTH PE: a	ARAC : 5 a amin GY: :	amino	o ac: id								
		(i:	i.) MOI	LECUI	LE TY	YPE:	inte	erna:	l fra	agmei	ıt					
30		(ж	i)seq	QUEN	CE DI	ESCR:	IPTI	ON: !	SEQ :	ID N	D: 5	:				
	Ser 1	Thr	Leu	Ser	Cys 5											
35	(7)	IN	FORM	ATIO	I FOR	R SE(Q ID	NO:	6 :							
40		(i)	, (I	4) LEI 3) TYI	VGTH:	: 15° amino	7 ami	ino a id		3			•			
40						3Y:]										
			L) MOI						200	FD 17						
45	Тиги									ID NO			2	_	_	_
	1	Phe			5					10			_		15	
		Gln		20			_		25		_			30		_
50		Thr	35		_	_	_	40				_	45			
		Ser 50					55			_	_	60				
	65	Val				70					75					80
55		Ser			85					90					95	
	ser	Asp	тте	TTG	rne	rne	GIU	Arg	ser	val	Pro	GIA	HIS	Asp	ASN	гуѕ

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Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
                                     120
                                                        125
       Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu
           130
                                135
                                                     140
       Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp
                             150
             INFORMATION FOR SEQ ID NO: 7:
        (8)
10
             (i) SEQUENCE CHARACTERISTICS:
                  (A) LENGTH: 157 amino acids
                   (B) TYPE: amino acid
                  (D) TOPOLOGY: linear
15
             (ii) MOLECULE TYPE: peptide
             (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
       Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn
20
       Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met
                                         25
        Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile
                35
                                     40
                                                          45
        Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser
25
                                                     60
       Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile 65 70 75 80
        Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser
                        85
                                             90
        Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu
30
                    100
                                         105
        Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu
               115
                                    120
                                                         125
       Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp
           130
                                135
                                                     140
        Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser
                             150
        (9) INFORMATION FOR SEQ ID NO: 8:
             (i) SEQUENCE CHARACTERISTICS:
                   (A) LENGTH: 471 base pairs
40
                   (B) TYPE: nucleic acid
                   (C) STRANDEDNESS: double
                  (D) TOPOLOGY: linear
             (ii) MOLECULE TYPE: cDNA
45
             (vi)ORIGINAL SOURCE:
                  (A) ORGANISM: human
                  (G) CELL TYPE: liver
             (ix) FEATURE:
50
                  (A) NAME/KEY: mat peptide
                  (B) LOCATION: 1..471
                  (C) IDENTIFICATION METHOD: E
             (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
55
       TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT
```

	Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu		Val	Ile	Arg	Asn		Asn	
	GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	10 AAT	CGG	ССТ	AT")	ملاطمك	15 (2) A	САТ	96
5	Asp	Gln	Val	Leu 20	Phe	Ile	Asp	Gln	Gly 25	Asn	Arg	Pro	Leu	Phe	Glu	Asp	, 36
	ATG	ACT	GAT	TCT	GAC	TGT	AGA	GAT		GCA	CCC	CGG	ACC		TTT	ATT	144
	Met	Thr	Asp 35	Ser	Asp	Cys	Arg	Asp	Asn	Ala	Pro	Arg		Ile	Phe	Ile	
	ATA	AGT		TAT	AAA	GAT	AGC		CCT	AGA	GGT	ATG	45 GCT	GTA	ACT	ATC	192
10	Ile	Ser 50	Met	Tyr	Lys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	Ala	Val	Thr	Ile	
	TCT	GTG	AAG	TGT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	TGT	GAG	AAC	AAA	ATT	240
	Ser 65	Val	гÀЗ	Cys	Glu	Lys 70	IIe	Ser	Thr	Leu	Ser 75	Cys	Glu	Asn	ГÀа	Ile 80	
	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
15	Ile	Ser	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr	Lys	
	AGT	GAC	ATC	ATA		TTT	CAG	AGA	AGT		CCA	GGA	CAT	GAT	95 AAT	AAG	336
	Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	330
	ΔТС	CAA	بلململ	100	тст	тсъ	TCA	тас	105	CCA	тъс	بكحلين	CTD	110	mor.	C	204
20	Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	384
			115					120					125		_		
	Lvs	GAG Glu	Arq	Asp	Leu	Phe	Lvs	Leu	Ile	Leu	Lvs	Lvs	GAG	Ago	GAA	TTG	432
		13	30				135					140					
25		GAT Asp															471
	145	p	9			150		****		GIII	155	GIU	ASD				
	(10) INE	FORM	TION	FOF	SEC) ID	NO:	9:								
30		(i)	SEQU	JENCE	CHA	RACT	ERIS	TICS	3 :								
			_) LEN					ids								
				3) TYI)) TOI													
		/ 4 4) MOT	РСТП	E 100	me.											
35			L) MOI						.1 6.		.						
			FRAC	21-11-114 1		E. I	1-ce1	. WIIIC		ayme	:110						
		(xi	L) SEÇ	QUENC	E DE	SCRI	PTIC	N: S	EQ I	D NC): 9:						
40	Met 1	Tyr	Phe	Gly	Lys 5	Leu	Glu	Ser	ГÀа	Leu 10	Ser						
	(11)) INE	ORMA	TION	I FOR	SEC) ID	NO:	10:								
		(;)	CEOT	IEMCE		חא מים	ים מקוי	·m T.C.C									
45		(1)	SEQU ()) LEN													
			(E	3) TYE	E: a	mino	aci	.d									
			(L) TOE	OLOG	Y: 1	.inea	r									
		(ii	L) MOI	ECUI	E TY	PE:	pept	ide									
50		(v)	FRAC	MENT	TYP	E: C	-ter	mina	l fr	agme	nt						
		(xi	L) SEC	UENC	E DE	SCRI	PTIC	N: S	EQ I	D NC	: 10):					
		Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp							
55	1				5					10							

	(12)	INFORMATION FOR SEQ ID NO: 11:
5		(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 13 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear
		(ii) MOLECULE TYPE: peptide
10		(v) FRAGMENT TYPE: N-terminal fragment
		(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:
15	Tyr 1	Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg 5 10
	(13)	INFORMATION FOR SEQ ID NO: 12:
20		(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 14 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear
		(ii) MOLECULE TYPE: peptide
		(v) FRAGMENT TYPE: internal fragment
25		(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
	Thr 1	tle Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg 5 10
30	(14)	INFORMATION FOR SEQ ID NO: 13:
35		(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 17 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear
		(ii) MOLECULE TYPE: peptide
		(v) FRAGMENT TYPE: internal fragment
40		(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
	Ile :	tle Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 5 10 15
45	(15)	INFORMATION FOR SEQ ID NO: 14:
50		(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 471 base pairs (B) TYPE: nucleic acid (C) STRANDEDNESS: double (D) TOPOLOGY: linear
		(ii) MOLECULE TYPE: cDNA
<i>55</i>		(ix) FEATURE: (A) NAME/KEY: mat peptide (B) LOCATION: 1471
		(C) IDENTIFICATION METHOD S

		(x:	L) SE(QUENC	CE DI	ESCR	[PTI	ON: 8	SEQ :	ED NO	D: 14	i :					
5	Tyr 1	TTT Phe	GIÀ	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu 15	Asn	48
	Asp	CAA Gln	Val	Leu 20	Phe	Ile	Asp	Gln	Gly 25	Asn	Arg	Pro	Leu	Phe 30	Glu	Asp	96
10	Met	ACT Thr	Asp 35	Ser	Asp	Ser	Arg	Asp 40	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	144
	Ile	AGT Ser 50	Met	Tyr	Lys	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	Ala	Val	Thr	Ile	192
15	Ser 65	GTG Val	Lys	Ser	Glu	Lys 70	Ile	Ser	Thr	Leu	Ser 75	Ala	Glu	Asn	Lys	Ile 80	240
	Ile	TCC	Phe	Lys	Glu 85	Met	Asn	Pro	Pro	Asp 90	Asn	Ile	Lys	Asp	Thr 95	Lys	288
20	Ser	GAC	Ile	Ile 100	Phe	Phe	Gln	Arg	Ser 105	Val	Pro	Gly	His	Asp 110	Asn	Lys	336
	Met	CAA Gln	Phe 115	Glu	Ser	Ser	Ser	Tyr 120	Glu	Gly	Tyr	Phe	Leu 125	Ala	Cys	Glu	384
25	Lys	GAG Glu 130	Arg	Asp	Leu	Phe	Lys 135	Leu	Ile	Leu	Lys	Lys 140	Glu	Asp	GAA	TTG Leu	432
<i>30</i>		GAT Asp															471
	(15)	INE	FORM.														
35		\-,	(<i>I</i>) LEN) TYP	GTH: E: a	10 mino	amin aci	o ac									
) MOI FRAC						ıl fr	acme	nt						
40			.) SEC									:					
	Tyr 1	Phe	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10							
45	(17)	INFO	RMAT SEQU			_											
50			() (E) (C)	LEN TYP STR TOP	GTH: E: n ANDE OLOG	471 ucle DNES Y: 1	basic a S: d inea	e pa cid loubl r	irs								,
		(11	.) MOI	ECUL	E TY	PE:	CDNA										

(ix)FEATURE:
(A)NAME/KEY: mat peptide
(B)LOCATION: 1..471

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

5	Tyr 1	TTT Phe	Gly	Lys	Leu 5	Glu	Ser	Lys	Leu	Ser 10	Val	Ile	Arg	Asn	Leu 15	Asn	48
10	GAC Asp	CAA Gln	GTT Val	CTC Leu 20	TTC Phe	ATT Ile	GAC Asp	CAA Gln	GGA Gly 25	AAT Asn	CGG Arg	CCT Pro	CTA Leu	TTT Phe 30	GAA Glu	GAT Asp	96
,,,	ATG Met	ACT Thr	GAT Asp 35	TCT Ser	GAC Asp	TCT Ser	AGA Arg	GAT Asp 40	AAT Asn	GCA Ala	CCC Pro	CGG Arg	ACC Thr 45	ATA Ile	TTT Phe	ATT Ile	144
15	Ile	AGT Ser 50	Met	Tyr	ГÀЗ	Asp	Ser 55	Gln	Pro	Arg	Gly	Met 60	Ala	Val	Thr	Ile	192
	Ser 65	GTG Val	Lys	Ser	Glu	Lys 70	Ile	Ser	Thr	Leu	Ser 75	Ala	Glu	Asn	Lys	Ile 80	240
20	ATT Ile	TCC Ser	TTT Phe	AAG Lys	GAA Glu 85	ATG Met	AAT Asn	CCT Pro	CCT Pro	GAT Asp 90	AAC Asn	ATC Ile	AAG Lys	GAT Asp	ACA Thr 95	AAA Lys	288
		GAC Asp													AAT		336
25		CAA Gln															384
	AAA Lys	GAG Glu 130	AGA Arg	GAC Asp	CTT Leu	TTT Phe	AAA Lys 135	CTC Leu	ATT Ile	TTG Leu	AAA Lys	AAA Lys 140	GAG Glu	GAT Asp	GAA Glu	TTG Leu	432
30		GAT Asp										GAA					471

(18) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 11464 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: genomic DNA
- (vi)ORIGINAL SOURCE:

 - (A)ORGANISM: human (G)CELL TYPE: placenta
- (ix) FEATURE:

35

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- (A) NAME/KEY: 5' UTR
- (B) LOCATION: 1..3
- (C) IDENTIFICATION METHOD: E
- (A) NAME/KEY: leader peptide (B) LOCATION: 4..82
- (C) IDENTIFICATION METHOD: S
 (A) NAME/KEY: intron
 (B) LOCATION: 83..1453

- (C) IDENTIFICATION METHOD: E
 (A) NAME/KEY: leader peptide
 (B) LOCATION: 1454..1465
- 55 (C) IDENTIFICATION METHOD: S

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(A) NAME/KEY: intron
(B) LOCATION: 1466..4848
                        (C) IDENTIFICATION METHOD: E
                        (A) NAME/KEY: leader peptide
                        (B) LOCATION: 4849..4865
                        (C) IDENTIFICATION METHOD: S
                        (A) NAME/KEY: mat peptide
                        (B) LOCATION: 4866..4983
                        (C) IDENTIFICATION METHOD: S
                        (A) NAME/KEY: intron
10
                        (B) LOCATION: 4984..6317
                        (C) IDENTIFICATION METHOD: E
                        (A) NAME/KEY: mat peptide
                        (B) LOCATION: 6318..6451
                        (C) IDENTIFICATION METHOD: S
                        (A) NAME/KEY: intron
15
                        (B) LOCATION: 6452..11224
                        (C) IDENTIFICATION METHOD: E
                        (A) NAME/KEY: mat peptide
                        (B) LOCATION: 11225..11443
                        (C) IDENTIFICATION METHOD: S
20
                        (A) NAME/KEY: 3' UTR
                        (B) LOCATION: 11444..11464
                        (C) IDENTIFICATION METHOD: E
                 (xi) SEQUENCE DESCRIPTION: SEO ID NO: 17:
          AAG ATG GCT GCT GAA CCA GTA GAA GAC AAT TGC ATC AAC TTT GTG GCA
                                                                                                        48
               Met Ala Ala Glu Pro Val Glu Asp Asn Cys Ile Asn Phe Val Ala
                     -35
                                                -30
                                                                           -25
          ATG AAA TTT ATT GAC AAT ACG CTT TAC TTT ATA G
                                                                           GTAAGG CTAATGCCAT
                                                                                                       98
          Met Lys Phe Ile Asp Asn Thr Leu Tyr Phe Ile Ala
30
                - 20
                                           -15
                                                                      -10
          AGAACAAATA CCAGGTTCAG ATAAATCTAT TCAATTAGAA AAGATGTTGT GAGGTGAACT
                                                                                                      158
         ATTAAGTGAC TCTTTGTGTC ACCAAATTC ACTGTAATAT TAATGGCTCT TAAAAAAATA
GTGGACCTCT AGAAATTAAC CACAACATGT CCCAAGGTCTC AGCACCTTGT CACACCACGT
GTCCTGGCAC TTTAATCAGC AGTAGCTCAC TCTCCAGGTT GCAGTAAGTG CACATCATGA
AAATCCCAGT TTTCATGGGA AAAATCCCAGT TTTCATTGGG AAAATCCCA
                                                                                                      218
                                                                                                      278
                                                                                                      338
                                                                                                      398
35
          GTACAAAACT GGGTGCATTC AGGAAATACA ATTTCCCAAA GCAAATTGGC AAATTATGTA
                                                                                                      458
          AGAGATTCTC TAAATTTAGA GTTCCGTGAA TTACACCATT TTATGTAAAT ATGTTTGACA AGTAAAAATT GATTCTTTTT TTTTTTTCT GTTGCCCAGG CTGGAGTGCA GTGGCACAAT
                                                                                                      518
                                                                                                      578
          CTCTGCTCAC TGCAACCTCC ACCTCCTGGG TTCAAGCAAT TCTCCTGCCT CAGCCTTCTG
                                                                                                      638
          AGTAGCTGGG ACTACAGGTG CATCCCGCCA TGCCTGGCTA ATTTTTGGGT ATTTTTACTA
                                                                                                      698
          GAGACAGGGT TTTGGCATGT TGTCCAGGCT GGTCTTGGAC TCCTGATCTC AGATGATCCT
                                                                                                      758
40
          CCTGGCTCGG GCTCCCAAAG TGCTGGGATT ACAGGCATGA ACCACCACAC ATGGCCTAAA AATTGATTCT TATGATTAAT CTCCTGTGAA CAATTTGGCT TCATTTGAAA GTTTGCCTTC
          ATTTGAAACC TTCATTTAAA AGCCTGAGCA ACAAAGTGAG ACCCCATCTC TACAAAAAAC TGCAAAATAT CCTGTGGACA CCTCCTACCT TCTGTGGAGG CTGAAGCAGG AGGATCACTT
                                                                                                      938
                                                                                                      998
          GAGCCTAGGA ATTTGAGCCT GCAGTGAGCT ATGATCCCAC CCCTACACTC CAGCCTGCAT
                                                                                                     1058
         GACAGTAGAC CCTGACACAC ACACACAAAA AAAAACCTTC ATAAAAAATT ATTAGTTGAC
TTTTCTTAGG TGACTTTCCG TTTAAGCAAT AAATTTAAAA GTAAAATCTC TAATTTTAGA
AAATTTATTT TTAGTTACAT ATTGAAATTT TTAAACCCTA GGTTTAAGTT TTATGTCTAA
ATTACCTGAG AACACACTAA GTCTGATAAG CTTCATTTTA TGGGCCTTTT GGATGATTAT
                                                                                                    1118
                                                                                                     1178
                                                                                                    1238
                                                                                                    1298
          ATAATATTCT GATGAAAGCC AAGACAGACC CTTAAACCAT AAAAATAGGA GTTCGAGAAA
                                                                                                    1358
         GAGGAGTAGC AAAAGTAAAA GCTAGAATGA GATTGAATTC TGAGTCGAAA TACAAAATTT TACATATTCT GTTTCTCTCT TTTTCCCCCT CTTAG CT GAA GAT GAT G GTAAA
                                                                                                    1418
50
                                                                                                    1470
                                                                Ala Glu Asp Asp Glu
                                                                -10
          GTAGAAATGA ATTTATTTTT CTTTGCAAAC TAAGTATCTG CTTGAGACAC ATCTATCTCA
                                                                                                    1530
          CCATTGTCAG CTGAGGAAAA AAAAAAATGG TTCTCATGCT ACCAATCTGC CTTCAAAGAA
                                                                                                    1590
          ATGTGGACTC AGTAGCACAG CTTTGGAATG AAGATGATCA TAAGAGATAC AAAGAAGAAC
                                                                                                    1650
55
          CTCTAGCAAA AGATGCTTCT CTATGCCTTA AAAAATTCTC CAGCTCTTAG AATCTACAAA 1710
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	ATAGACTTTG	CCTGTTTCAT	TGGTCCTAAG	ATTAGCATGA	AGCCATGGAT	TCTGTTGTAG	1770
	GGGGAGCGTT	GCATAGGAAA	AAGGGATTGA	AGCATTAGAA	TTGTCCAAAA	TCAGTAACAC	1830
	CTCCTCTCAG	AAATGCTTTG	GGAAGAAGCC	TGGAAGGTTC	CGGGTTGGTG	GTGGGGTGGG	1890
	GCAGAAAATT	CTGGAAGTAG	AGGAGATAGG	AATGGGTGGG	GCAAGAAGAC	CACATTCAGA	1950
5	GGCCAAAAGC	TGAAAGAAAC	CATGGCATTT	ATGATGAATT	CAGGGTAATT	CAGAATGGAA	2010
	GTAGAGTAGG	AGTAGGAGAC	TGGTGAGAGG	AGCTAGAGTG	ATAAACAGGG	TGTAGAGCAA	2070
	GACGTTCTCT	CACCCCAAGA	TGTGAAATTT	GGACTTTATC	TTGGAGATAA	TAGGGTTAAT	2130
	TAAGCACAAT	ATGTATTAGC	TAGGGTAAAG	ATTAGTTTGT	TGTAACAAAG	ACATCCAAAG	2190
	ATACAGTAGC	TGAATAAGAT	AGAGAATTTT	TCTCTCAAAG	AAAGTCTAAG	TAGGCAGCTC	2250
	AGAAGTAGTA	TGGCTGGAAG	CAACCTGATG	ATATTGGGAC	CCCCAACCTT	CHICAGTCTT	2310
10	GTACCCATCA	TCCCCTAGTT	GTTGATCTCA	CTCACATAGT	TGAAAATCAT	CATACTTCCT	2370
	CCCTTCATAT	CCCAGTTATC	AAGAAAGGGT	CAAGAGAAGT	CAGGCTCATT	CUINCIICCI	
	ACTCTA ATTC	CAACTTAAAC	ACATCAATCC	CCCTCATATA	CCATTGACTA	CCITICAMAG	2430
	ACICIAATIG	DOCADOTIONS	ACCI A A TOTO	COCIUMIAII	TCTTTATTCC	GAATTTAATC	2490
	ACAI GGCCAC	ANNAMEDICA	AGGAMAICIG	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	TCATTCTGGC	AGGTAGCCAT	2550
	AIGACICIII	COCOCCCCO	AMIMAIAIAI	1111AAAATA	TCATTCTGGC	TTTGGTATAA	2610
15	AGAATIGATG	GIGIGGGGIG	AGGAGGCCAA	AATTAAGGGT	TGAGAGCCTA	TTATTTTAGT	2670
	TATTACAAGA	AATGATGGTG	TCATGAATTA	AGGTAGACAT	AGGGGAGTGC	TGATGAGGAG	2730
	CTGTGAATGG	ATTTTAGAAA	CACTTGAGAG	AATCAATAGG	ACATGATTTA	GGGTTGGATT	2790
	TGGAAAGGAG	AAGAAAGTAG	AAAAGATGAT	GCCTACATTT	TTCACTTAGG	CAATTTGTAC	2850
	CATTCAGTGA	AATAGGGAAC	ACAGGAGGAA	GAGCAGGTTT	TGGTGTATAC	AAAGAGGAGG	2910
	ATGGATGACG	CATTTCGTTT	TGGATCTGAG	ATGTCTGTGG	AACGTCCTAG	TGGAGATGTC	2970
20	CACAAACTCT	TCTACATGTG	GTTCTGAGTT	CAGGACACAG	ATTTGGGCTG	GAGATAGAGA	3030
	TATTGTAGGC	TTATACATAG	AAATGGCATT	TGAATCTATA	GAGATAAAAA	GACACATCAG	3090
	AGGAAATGTG	TAAAGTGAGA	GAGGAAAAGC	CAAGTACTGT	GCTGGGGGGA	ATACCTACAT	3150
	TTAAAGGATG	CAGTAGAAAG	AAGCTAATAA	ACAACAGAGA	GCAGACTAAC	CAAAAGGGGA	3210
	GAAGAAAAAC	CAAGAGAATT	CCACCGACTC	CCAGGAGAGC	ATTTCAAGAT	TGAGGGGATA	3270
	GGTGTTGTGT	TGAATTTTGC	AGCCTTGAGA	ATCAAGGGCC	AGAACACAGC	TTTTAGATTT	3330
25	AGCAACAAGG	AGTTTGGTGA	TCTCAGTGAA	AGCAGCTTGA	TGGTGAAATG	GAGGCAGAGG	3390
	CAGATTGCAA	TGAGTGAAAC	AGTGAATGGG	AAGTGAAGAA	ATGATACAGA	TAATTCTTGC	3450
	TAAAAGCTTG	GCTGTTAAAA	GGAGGAGAGA	AACAAGACTA	GCTGCAAAGT	GAGATTGGGT	3510
	TGATGGAGCA	GTTTTAAATC	TCAAAATAAA	GAGCTTTGTG	CTTTTTTGAT	TATGAAAATA	3570
	ATGTGTTAAT	TGTAACTAAT	TGAGGCAATG	AAAAAAGATA	ATAATATGAA	חת את את אתם מ	3630
30	ATAAAAACCA	CCCAGAAATA	ATGATAGCTA	CCATTTTCAT	ACAATATTTC	TOTAL TANGET	3690
30	TCTATGTATA	TATACAGACA	CAGAAATGCT	עודיניטוייויי עידעיי	TTAAAAGGGA	TACACICCII	3750
	CCTAAGCTGC	THETHUCTACT	TACTCATATA	TATGGACATC	TCTCCATGGC	AUCCUCHAU	
	TGCAGTTATA	TTANGTTCAT	CATATTTCAC	AATAACCCCA	TATCTTTGCC	AACGAGTAAT	3810
	AATCAATTATA	TARTUCCUCA	AUCOUNTACTOR	CCACMMTTCM	GTTGTTATTA	CITITIATT	3870
	CATAACCATT	CCTCTACACC	ANTOTTOTT	CAGILIGII	ATTTTTTCTT	ACAATGTTCC	3930
35	CCCACCACCT	ACA A TOTAL COTO	CCDECTORACA	ACTORAGE	TGATTGCCAA	CAGGATAAAA	3990
55	CACTACACCC	TACATCCCCA	CCACAAATCC	AGAGAAAGGA	AGATACCAGA	ATTAAAGCTT	4050
	CAGIAGAGGG	COMMOGGA	GCACAAAIGG	GATCAGCCCT	AGATACCAGA	AATGGCACTT	4110
	TOTCATITUE	CCTTGGGACA	AAAGGGAGAG	AGGCAATAAC	TGTGCTGCCA	GAGTTAAATT	4170
	TGTACGTGGA	GTAGCAGGAA	ATCATTTGCT	GAAAATGAAA	ACAGAGATGA	TGTTGTAGAG	4230
	GTCCTGAAGA	GAGCAAAGAA	AATTTGAAAT	TGCGGCTATC	AGCTATGGAA	GAGAGTGCTG	4290
40	AACTGGAAAA	CAAAAGAAGT	ATTGACAATT	GGTATGCTTG	TAATGGCACC	GATTTGAACG	4350
	CTTGTGCCAT.	TGTTCACCAG	CAGCACTCAG	CAGCCAAGTT	TGGAGTTTTG	TAGCAGAAAG	4410
	ACAAATAAGT	TAGGGATTTA	ATATCCTGGC	CAAATGGTAG	ACAAAATGAA	CTCTGAGATC	4470
	CAGCTGCACA	GGGAAGGAAG	GGAAGACGGG	AAGAGGTTAG	ATAGGAAATA	CAAGAGTCAG	4530
	GAGACTGGAA	GATGTTGTGA	TATTTAAGAA	CACATAGAGT	TGGAGTAAAA	GTGTAAGAAA	4590
	ACTAGAAGGG	TAAGAGACCG	GTCAGAAAGT	AGGCTATTTG	AAGTTAACAC	TTCAGAGGCA	4650
45	GAGTAGTTCT	GAATGGTAAC	AAGAAATTGA	GTGTGCCTTT	GAGAGTAGGT	TAAAAAACAA	4710
	TAGGCAACTT	TATTGTAGCT	ACTTCTGGAA	CAGAAGATTG	TCATTAATAG	TTTTAGAAAA	4770
	CTAAAATATA	TAGCATACTT	ATTTGTCAAT	TAACAAAGAA	ACTATGTATT	TTTAAATGAG	4830
	ATTTAATGTT	TATTGTAG A	A AAC CTG G	AA TCA GAT	TAC TTT GGC	AAG CTT	4880
		Gl	u Asn Leu G	lu Ser Asp	Tyr Phe Gly	Lvs Leu	
		-	-5	-	1	5 5	
50	GAA TCT AAZ	TTA TCA GI		AT TTG AAT	GAC CAA GTT		4928
	Glu Ser Lvs	Leu Ser Va	l Ile Arg A	sn Len Asn	Asp Gln Val	Leu Dhe	* J Z O
		10		15	OTH VOI	20	
	ATT GAC CAR		בב כיכידי כידים יד		ATG ACT GAT		4076
	The Asn Gir	Gly ben by	o Dro Leu B	he Glu Nam	Met Thr Asp	LCI GAC	4976
	TIC HOD GII	25 ASII AI				ser wab	
55	TGT AGA G			0	35	COMPONE SASE	
	IGI AGA G	GIMILLIT	TIMMITCOCA	AACA LAGAAA	TGACTAGCTA	CITCITCCCA	5032

	Cys Arg Asp	
	40	
	TTCTGTTTTA CTGCTTACAT TGTTCCGTGC TAGTCCCAAT CCTCAGATGA AAAGTCACAG	5092
	GAGTGACAAT AATTTCACTT ACAGGAAACT TTATAAGGCA TCCACGTTTT TTAGTTGGGG	5152
5	TAAAAAATTG GATACAATAA GACATTGCTA GGGGTCATGC CTCTCTGAGC CTGCCTTTGA	
	ATCACCAATC CCTTTATTGT GATTGCATTA ACTGTTTAAA ACCTCTATAG TTGGATGCTT	5212
	AATCCCTGCT TGTTACAGCT GAAAATGCTG ATAGTTTACC AGGTGTGGTG GCATCTATCT	5272
	GTAATCCTAG CTACTTGGGA GGCTCAAGCA GGAGGATTGC TTGAGGCCAG GACTTTGAGG	5332
	GRANICETAG CIACITIGGGA GGCICAAGCA GGAGGATIGC TIGAGGCCAG GACTITGAGG	5392
	CTGTAGTACA CTGTGATCGT ACCTGTGAAT AGCCACTGCA CTCCAGCCTG GGTGATATAC	5452
10	AGACCTTGTC TCTAAAATTA AAAAAAAAAA AAAAAAAAAC CTTAGGAAAG GAAATTGATC	5512
	AAGTCTACTG TGCCTTCCAA AACATGAATT CCAAATATCA AAGTTAGGCT GAGTTGAAGC	5572
	AGTGAATGTG CATTCTTTAA AAATACTGAA TACTTACCTT AACATATATT TTAAATATTT	5632
	TATTTAGCAT TTAAAAGTTA AAAACAATCT TTTAGAATTC ATATCTTTAA AATACTCAAA	5692
	AAAGTTGCAG CGTGTGTTT GTAATACACA TTAAACTGTG GGGTTGTTTG TTTGTTTGAG	5752
	ATGCAGTTTC ACTCTGTCAC CCAGGCTGAA GTGCAGTGCA	5812
15	CTCACTACAA CCTCCACCTC CCACGTTCAA GCGATTCTCA TGCCTCAGTC TCCCGAGTAG	5872
	GTGGGATTAC AGGCATGCAC CACTTACACC CGGCTAATTT TTGTATTTTT AGTAGAGCTG	
	GGGTTTCACC ATGTTGGCCA GGCTGGTCTC AAACCCCTAA CCTCAAGTGA TCTGCCTGCC	5932
	TCAGCCTCCC AAACAAACAA ACAACCCCAC AGTTTAATAT GTGTTACAAC ACACATGCTG	5992
	CARCUTATION OF THE PROPERTY AND THE PROPERTY OF THE PROPERTY O	6052
	CAACTTTTAT GAGTATTTA ATGATATAGA TTATAAAAGG TTGTTTTTAA CTTTTAAATG	6112
20	CTGGGATTAC AGGCATGAGC CACTGTGCCA GGCCTGAACT GTGTTTTTAA AAATGTCTGA	6172
20	CCAGCTGTAC ATAGTCTCCT GCAGACTGGC CAAGTCTCAA AGTGGGAACA GGTGTATTAA	6232
	GGACTATCCT TTGGTTAAAT TTCCGCAAAT GTTCCTGTGC AAGAATTCTT CTAACTAGAG	6292
	TTCTCATTTA TTATATTTAT TTCAG AT AAT GCA CCC CGG ACC ATA TTT ATT	6343
	Asp Asn Ala Pro Arg Thr Ile Phe Ile	
	40 45	
	ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA GGT ATG GCT GTA ACT ATC	6391
25	Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile	0331
	TCT GTG AAG TGT GAG AAA ATT TCA ACT CTC TCC TGT GAG AAC AAA ATT	
	Cor Val Lya Cya Clu Lya Tla Con Wha Lya Car Gar GAG AAC AAA ATT	6439
	Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile	
	65 70 75 80	
30	ATT TCC TTT AAG GTAAG ACTGAGCCTT ACTTTGTTTT CAATCATGTT AATATAATCA	6496
	Ile Ser Phe Lys	
	ATATAATTAG AAATATAACA TTATTTCTAA TGTTAATATA AGTAATGTAA TTAGAAAACT	
		6556
	CAAATATCCT CAGACCAACC TTTTGTCTAG AACAGAAATA ACAAGAAGCA GAGAACCATT	
	CAAATATCCT CAGACCAACC TTTTGTCTAG AACAGAAATA ACAAGAAGCA GAGAACCATT AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT	6616
	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT	6616 6676
35	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCTGA	6616 6676 6736
35	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT GTGAGTTATA CATTTAAGAA	6616 6676 6736 6796
35	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT GTGAGTTATA CATTTAAGAA TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTTAATT ATCCTTCTAT	6616 6676 6736 6796 6856
35	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT GTGAGTTATA CATTTAAGAA TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC TAGTTGTTTT GTTGCTGATC	6616 6676 6736 6796 6856 6916
35	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT GTGAGTTATA CATTTAAGAA TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC TAGTTGTTTT GTTGCTGATC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTATGTTA TTTTTAATCT	6616 6676 6736 6796 6856 6916 6976
35	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT GTGAGTTATA CATTTAAGAA TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC TAGGTGATC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTATGTTA TTTTTAATGT TAATCTAATT GAATAAAAGT TATGAGAATCA GCTGTAAAAG TAATGCTATA ATTATCTTCA	6616 6676 6736 6796 6856 6916 6976 7036
<i>35</i>	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT GTGAGTTATA CATTTAAGAA TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC TAGGTTGAT GTTGCTGATC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTATGTTA TTTTTAATGT TAATCTAATT GAATAAAAGT TATGAGAATCA GCTGTAAAAG TAATGCTATA ATTATCTTCA AGCCAGGTAT AAAGTATTTC TGGCCTCTAC TTTTTCTCTA TTATTCTCCA TTATTATTCT	6616 6676 6736 6796 6856 6916 6976 7036 7096
	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT GTGAGTTATA CATTTAAGAA TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC TAGTTGTTT GTTGCTGATC CTTAGCCTAA GAATAAAAGT TATGAGATCA GCTGTAAAAG ATGATGTTA TTTTTTAATGT TAATCTAATT GAATAAAAGT TATGAGATCA GCTGTAAAAG TAATGCTATA ATTATCTTCA CTATTATTTT TCTCTATTTC CTCCATTATT GTTAGATAAA CCACAATTAA CTATAGCTAC	6616 6676 6736 6796 6856 6916 6976 7036
	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT GTGAGTTATA CATTTAAGAA TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC TAGTTGTTT GTTGCTGATC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTATGTTA TTTTTAATGT TAATCTAATT GAATAAAAGT TATGAGATCA GCTGTAAAAG TAATGCTATA ATTATCTTCA AGCCAGGTAT AAAGTATTTC TGGCCTCTAC TTTTTCTCTA TTATTCTCCA TTATTATTCT CTATTATTTT TCTCTATTTC CTCCATTATT GTTAGATAAA CCACAATTAA CTATAGCTAC AGACTGAGCC AGTAAGAGTA GCCAGGGATG CTTACAAAATT GGCAATGCTT CAGAGGGAGA	6616 6676 6736 6796 6856 6916 6976 7036 7096
	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT GTGAGTAAT CATTTAAGAA TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC TAGTTGTTTT GTTGCTGATC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTATGTTA TTTTTAATGT TAATCTAATT GAATAAAAGT TATGAGATCA GCTGTAAAAG TAATGCTATA ATTATCTTCA AGCCAGGTAT AAAGTATTC TGGCCTCTAC TTTTTCTCTA TTATTCTCA TTATTATCT CTATTATTTT TCTCTATTTC CTCCATTATT GTTAGATAAA CCACAATTAA CTATAGCTAC AGACTGAGCC AGTAAGAGTA GCCAGGGATG CTTACAAATT GGCAATGCTT CAGAGGGAA TTCCATGTCA TGAAGACTCT TTTTGAGTGG AGATTTGCCA ATAAATATCC GCCTTTCATGC	6616 6676 6736 6796 6856 6916 6976 7036 7096 7156
	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC TAGTTGTTTT GTTGCTGATC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTATGTTA TTTTTAATGT TAATCTAATT GAATAAAAGT TATGAGATCA GCTGTAAAAG TAATGCTATA ATTATCTTCA AGCCAGGTAT AAAGTATTC TGGCCTCTAC TTTTTCTCTA TTATTCTCCA TTATTATTCT CTATTATTTT TCTCTATTTC CTCCATTATT GTTAGATAAAT TATTTCTCCA AGACTGAGCC AGTAAGAGTA GCCAGGGTG CTTACAAATTT GGCAATGCTT CAGAGGGAGAA TTCCATGTCA TGAAGACTCT TTTTGAGTGG AGATTTGCCA ATGAAGGTA CCACAGGGCA CCACCCAGTC CCCACTGAAA GACAGTTAGG ATATGACCTT AGTGAAGGTA CCACAGGGCA	6616 6676 6736 6796 6856 6916 6976 7036 7096 7156 7216
	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTTATACAAA TAATAATGA GAATACATAT ATACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC TAGTTGTTTT GTTGCTGATC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTATATTA TTTTTAATGT TAATCTAATT GAATAAAAGT TATGAGATCA GCTGTAAAAG TAATGCTATA ATTATCTCA AGCCAGGTAT AAAGTATTTC TGGCCTCTAC TTTTTCTCTA TTATTCTCCA TTATTATTCT CTATTATTTT TCTCTATTTC CTCCATTATT GTTAGATAAA CCACAATTAA CTATAGCTAC AGACTGAGCC AGTAAGAGTA GCCAGGGATG CTTACAAATT GGCAATGCTT CAGAGGAGAA TTCCATGTCA TGAAGACTCT TTTTGAGTGG AGATTTGCCA AGTAAGAGTA CCACAGGGCCA ACTTGGTAGG GAGAAAAAAA CCCACTCTAAA ATATAATCCA AGTAAGACA GTGCATATGC	6616 6676 6736 6796 6856 6916 7036 7096 7156 7216 7276 7336
	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTATACAAA TAATAATGTA GAATACATAT TAATCAGAC TTTATACAAA TAATAATGTA GAATACATAT TATCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC CTTAGCCTAA GAATAAAAAGT TATGATAATA TTTTAATCCC CTTAGCCTAA GAATAAAAAGT TATGAGATCA GCTGTAAAAG TAACCTAATT GAATAAAAAGT TATGAGATCA GCTGTAAAAG AGCCAGGTAT AAAGTATTC TGGCCTCTAC TTTTTCTCTA TTATTCTCCA TTATTTCT CTATTATTT TCTCTATTTC CTCCATTATT GTTAGATAAA CCACAAATTA CTATAGCTAC AGACTGAGCC AGTAAGAGTA GCCAGGGATG CTTACAAATT GGCAATGCTT CAGAGGAGAA TTCCATGTCA TGAAGACTCT TTTTGAGTGG AGATTTGCCA ATAAATATCC GCTTTCATGC CCACCCAGTC CCCACTGAAA GACAGTTAGG ATAATACCCA AGTAAGAACA GTGCATATGC AACAGATACA GCCCCCAGAC AAATCCCTCA GCTATCTCCC TCCAACCAGA GTGCCACCCC	6616 6676 6736 6796 6856 6916 6976 7036 7036 7156 7216 7276 7336 7396
40	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTATACAAA TAATAATGTA GAATACATAT TAATCAGAC TTTATACAAA TAATAATGTA GAATACATAT TATCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC CTTAGCCTAA GAATAAAAAGT TATGATAATA TTTTAATCCC CTTAGCCTAA GAATAAAAAGT TATGAGATCA GCTGTAAAAG TAACCTAATT GAATAAAAAGT TATGAGATCA GCTGTAAAAG AGCCAGGTAT AAAGTATTC TGGCCTCTAC TTTTTCTCTA TTATTCTCCA TTATTTCT CTATTATTT TCTCTATTTC CTCCATTATT GTTAGATAAA CCACAAATTA CTATAGCTAC AGACTGAGCC AGTAAGAGTA GCCAGGGATG CTTACAAATT GGCAATGCTT CAGAGGAGAA TTCCATGTCA TGAAGACTCT TTTTGAGTGG AGATTTGCCA ATAAATATCC GCTTTCATGC CCACCCAGTC CCCACTGAAA GACAGTTAGG ATAATACCCA AGTAAGAACA GTGCATATGC AACAGATACA GCCCCCAGAC AAATCCCTCA GCTATCTCCC TCCAACCAGA GTGCCACCCC	6616 6676 6736 6796 6856 6976 7036 7096 7156 7216 7236 7336 7396
40	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACATCAGTC TTTATACAAA TAATAATGTA GAATACATAT TTATGACCTG CATCTCCTGA TACACAGTGTGA CATTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC TAGTTGTTT GTTGGTGATC CTTAGCCTAA GCCTGAAAAGT TATGAGATCA GCTGTAAAAG TTATGATTATT TAGGCTTCTA TTTTTTCTCTA TTATTCTCTATTTCTTATTTT TCTCTATTTC CTCCATTATT GTTAGATAAA CCACAATTAA CTATAGCTAC AGACTGAGCC AGTAAGAGTA GCCAGGGATG CTTACAAATT GCACAATTAA CTATAGCTAC CCACCAGTC CCCACCAGAA GACAGATAGA GACAGTTAGG ATATGACCTC AGTGAAGACA GTCCACACCCC TCCAGCGAC AAATCCCTCA GACCTGACA GTGCACCCC TCCAGCTGAC GCCCCCCCCCC	6616 6676 6776 6796 6856 6976 7036 7096 7156 7216 7276 7336 7336 7456 7516
40	AAAGTGAATA CTTACTAAAA ATTATCAAAC CTTTTACCTA TTGTGATAAT CAACGGGAA ACAATCAGTC CACAGGGGAA ACAATCAGTC TTATACAAA TAATAATGTA ACAATCAGTC TTATACAAA TAATAATGTA ATTATCAAC CTTTCCAGAA TAGATACATA TATTCTACAC CTTTGTAAAT TATGATAATA TATTCTACAC CTTTGTAAAT TATGATAATA TATTCTACAC CTTTGTAAAT TATGATAATA TATGATAATA TATTATCC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG TAATCTAATT CAAAGATATT CTAGTATTT CTCTATTTC CTCCATTATT CTCTATTTC CTCCATTATT CTCATTATT TCTCATTTC CTCCATGAC AGACCAGCC CCCCCAGTC CCCCCCAGC CCCCCAGC ACTTGGAGC CCCCCCAGC CCCCCCAGC ACTTGGAGC CCCCCCAGC CCCCCAGC CCCCCCAGC CCCCCAGC CCCCCAGC CCCCCCAGC CCCCCAGC CCCCCAGC CCCCCAGC CCCCCAGC CCCCCAGC CCCCCAGC CCCCCAGC CCCCCCAGC CCCCCCAGC CCCCCCAGC CCCCCCAGC CCCCCCAGC CCCCCCAGC CCCCCCCC	6616 6676 6736 6796 6856 6916 7036 7036 7156 7216 7276 7336 7456 75516 7576
40	AAAGTGAATA CTTACTAAAA ATTATCAAAC CTTTTACCTA TTGTGATAAT GATGGTTTT CTGAGCCTGT CACAGGGGAA ACAGGAGATAC ACACTTGTT TTATACAAA TAATAATGTA ACAATCAGTC TTTATACAAA TAATAATGTA AAAACATTGTA TTATACAAA TAATAATGTA AAAACATTGTA TTATACAAA TAATAATGTA AAAACATTATT ATTCTACAC CTTTGTAAAT TATGATAATA TATTATAATCC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG TAATCTAATT GAATAAAAGT TATGAGATCA GCCAGGTAT AAAGTATTC CTCATTATT CTCTATTTC CTCCATTATT CTCTATTTC CTCCATTATT TCTCATTTC CTCCATTATT TCTCATTTC CTCCATTATT TCTCATTTC CTCCATTATT TCTCATTTC CTCCATTATT TCTCATTTC CTCCATTATT TCTCATTTC CCCACCAGTC AGAAGACTCT TTTTGAGTGG AGATTAGCC CCCACCGAC ACTTGGAGC ACTTGGAGC ACTTGGAGC ACTTGGAGC ACTTGGAGC AATAAAAAG CCCCCAGAC AAATCCCTCA AATAAATACC CCCACCAGAC AATTCCCCC TTCAGGTGAC AATTTGGAGG CCCCCAGAC AACACTCTAAA ATATAATCCA AGTGAAGGTA CCCAACTCAC GCCACTGAC AACACTACA CCCCAGTC TTCAGGTGAC CCCCCAGAC AACACCCCC TTCAGGTGAC AATTTGGAGC CCCCCAGAC AACACCCCC TTCAGGTGAC AACACAGAT AACACTTACC TTCAGGTGAC AATTTGGAGC AACACTACAC AACACACAC	6616 6676 6736 6796 6856 6916 7036 7036 7216 7216 7276 7336 7456 7576 7636
40	AAAGTGAATA CTTACTAAAA ATTATCAAAC CTTTTACCTA TTGTGATAAT CACAGGGGAA ACACTTGTT TTATACAAA ATAAATGTA ACAATCAGTC TTTATACAAA TAATAATGTA ACAATCAGTC TTTATACAAA TAATAATGTA AGATCAGTC TTTATACAAA TAATAATGTA AGATCAGTC TTTATACAAA TAATAATGTA AGATCAGTC CTTTCCAGAA TGAGTTCTC TATGAAGAAT ATCCTTCTAT ATTCTACAC CTTTGTAAAT TATGATAATA TATGATAATA TTTTATATCC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTGTTTT TAATCTAATT GAATAAAAGT TATGAGATCA GCCCCGAGTA AGACTATT TCTCTATTTC CTCATTATT TCTCTATTTC CTCATTATT TCTCATTTC AGAGAGATA AGACTCTCAG AGACTGAGC AGTAGAGATA GCCAGGGATG CCCACCAGTC ACCCCAGTC CCCACTGAAA GACAGTTAGG ACATTAGCTA ATAATATCC AGAGGGAAA ACACTGGATC ACACAATTAA CCACAATTAA CTATATCT CAGAGGGAA ACACTTAGG AGACAGATAC CCCACCAGAC AAATCCCTCA ACACAATAA ATAATATCC CCCACCAGAC AAATCCCTCA ACCTGAACA CCCCCAGAC AAATCCCTCA GCCACTGACA ACCTGAACA CCCCCAGAC AAATCCCTCA ACCTGACAG CCCCCAGAC AACATAATA CCCCCACTGAA ATATCCCCC CCCCACTGAC AACATAATAC CCCCCAGAC AACACATAAT CCCCCCAGAC AACACACAG CCCCCAGAC AACACACAC	6616 6676 6736 6796 6856 6916 7036 7096 7156 7276 7336 7396 7456 7516 7576 7636
40 45	AAAGTGAATA CTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTT CTGAGCCTGT CACAGGGGAA GAGAGATACA AACACTTGTT TTATGACCTG CATCTCCTGA TACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT ATTTCTACAC CTTTGTAAAT TATGATAATA TTTTAATCCC CTTAGCCTAA GATCTTAGACA CAAGCTTCAG CTTCCAGTTG TAATCTAATT GAATAAAAGT TATGAGATCA GCTGTAAAAG AGCCAGGTAT AAAGTATTC TGGCCTCTAC TTTTTCTCTA TTATTCTCA TATTTCTCA AGCCAGGTAT TCCTATTTC CTCCATTATT GTTAGATAAA CCACAAATTA ATTATCTTC CTATTATTTT TCTCTATTTC CTCCATTATT GTTAGATAAA CCACAAATTA CTATTATCT CCACCAGTC AGAGACTCT TTTTGAGTGG AGATTTGCCA ACACCAGTC CCCACTGAAA GACAGTTAGG ATATGACCTA AGTAAAACA ACCTGGAGC CCCCCAGAC AAATCCCTCA GCTATCTCC TCCAACCAGA GTGCATATGC AACAGATACA GCCCCCAGAC AAATCCCTCA GCTATCTCC TCCAACCAGA GTGCCACCCC TTCAGGTGAC AATTTGGAGT CCCCCATTCTA GACCTGACAG GTGCCACCCC TTCAGGTGAC AATTTGGAGT AGGGAAAACA GTGCATATGC AACATAATTA GAAGGAAGG AGAGGGGGA AGCTCAAGCTA TTATCAAAATA GCATAAGAG CCCCCAGAC AAACCCCCA GCTACACGTA TTATCAAAATA GCATAAGAG CCCCCAGAC AAACCCCCA GCTACACGTA TTATCAAAATA GCATAAATTA GAAGGAAGG AGATGCCAA GCTCAAGCTA TGTGGGATAG AGGAAAACCC ACCCAGGA GCGAATTCAG AAACTCGCAA GCTCAAGCTA TGTGGGATAG AGGAAAACCC TCCAGGAGG AAACCCGCA AACCTCAAGCTA TGTGGGATAG AGGAAAACCC TCCAGGAGG AAACCGGGAT AAGTCCGAAC CTACAGGTG ATTCTTGTTG AGCGAGACTG GTGAAAATGT TAAGAAGATG GAAAATATGC TTAGGGAACCCCC TTCAGGTGA GCAGATTCAG AAACTCGGGAT AAGTCCGAAC CTACAGGTG ATTCTTGTTG AGCGGAGACTG GTGAAAATGT TAAGAAGATG GAAAATATGC TTGGGGATAG AGGAAAACCC AGCGGAGACTG GTGAAAATGT TAAGAAGATG GAAAATATGC TTGGGATAG AGGAAAACCC AGCGGAGACTG GTGAAAATGT TAAGAAGATG GAAAATATATC TTGGCACTTA GTTAGGAACTC AGCGGAGACTG GTGAAAATGT TAAGAAGATG GAAAATATATGC TTGGGAACTG TTGTGGGATAG AGGAAAACCC AGCGGAGACTG GTGAAAATGT TAAGAAGATG GAAAATATATGC TTGGGAACTT TTGTTTTGCAACCTTA GTTAGAACTT TTGGCACTTA GTTAGGAACTC AGCGGAGACTG GTGAAAATGT TAAGAAGATG GAAAATATATG TTGGCACTTA GTTAGGAACTC AGCGGAGACTG GTGAAAATGT TAAGAAGATG GAAAATATATG TTGGCACTTA GTTAGGAACTC AGGGAGAACTG GTGAAAATGT TAAGAAGATG GAAAATATATG TTGGCACTTA GTTAGGAACTC	6616 6676 6736 6796 6856 6916 6976 7036 7156 7216 7336 7396 7516 7536 7636 7756
40	AAAGTGAATA CTACTAAAA ATTATCAAAC TCTTTACCTA CTGAGCCTGT CACAGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA ACAATCAGTC TTATACAAA TAATAATGTA GAATACATAT TAATCAGTC CTTTCCAGAA TGAGTTCTGC TATGAAGAAT ATTTCTACAC CTTTGTAAAT TATGATAATA TATGATAATA TATGATAATA TATGATAATA TATGATAATA TATGATAATA TATGATAATA TTTTAATCCC CTTAGCCTAA GTCTTAGACA GCAGGTTCAG CTTCCAGTTG TAATCTTATT GAATAAAAGT TATGAGATCA GCTGTAAAAA GCCAGGTAT TACTCATTC CTATTATTT TCTCTATTTC CTCATTTC CTCATTATT TCTCTATTC CTCCATTATT TCTCTATTC CTCCATTATT TTTTGAGTGG AGAATAAAA GCCAGGATG TTCCATGTCA TGAAGACTCT TTTTGAGTGG AGAATACA ACTTGGTAG ACTTGGTAG ACCTCTAAA ACTTGGTAG ACCTCTAAA ATATATCCC TCCACCAGTC TCCAGCCA ACCTCTAAA ATATATCCC TCCACCAGTC TCCAGCCA AAATCCCTCA AAATCCCTCA ACCTGCAAA AAATCCCTCA ACCTGCAAA AAATCCCCCA ACCACAATTAA CCACAATTAA ACTAGGGCA AAATCCCCCA ACCACAATTAA ACTAGAGACA ACCTCTAAA ATATAATCC CCACCAGAC AAATCCCCCA ACCACAATTAA ACTAGAGACA ACCTCTAAA ATATAATCCC TCCAACCAGA ACCACGATAC ACCTCCAGAC AAATCCCCCA ACCACAATTAA ACTAGAGACA ACCTCCAAA ACCTGCACA ACCTCCAAA ACCTGCACAC ACCACAATTAA ACTAGAGACA ACCTCCAAA ACCTGCACAC ACCACAATTAA ACTAGAGACA ACCTCCAAA ACCTGCACAC ACCACAATTAA ACTAGAGACA ACCTCCAAA ACCTGCACAC ACCACAATTAA ACCACTCTAA ACCTCCAGCC ACCACAATTAA ACCACACTTCAA ACCTCCAGCC ACCACAATTAA ACCACCACT ACCACCACAA ACCTCCACCAC ACCACAATTAA ACCACCACAATTAA ACCACCACT ACCACCACAA ACCACCACT ACCACCACAA ACCACCACAATTAA ACCACCACAATTAA ACCACCACAAATAA ACCACCACAATTAA ACCACCACAATTAA ACCACCACAATTAA ACCACCACAAATAA ACCACCACAAATAA ACCACCACAAATAAA ACCACCACCAC ACCACCACAAATAA ACCACCACAAATAAAAAA ACCACCACAAATAAAAAA ACCACCACCAC ACCACCACAAATAA ACCACCACAAATAAAAAA ACCACCACAAATAAAAAAA ACCACCACAAATAAAAAAA ACCACCACAAAAAAAA	6616 6676 6736 6796 6856 6916 6976 7036 70156 7216 7216 7336 7456 7556 7556 7756 7756 7756
40 45	AAAGTGAATA CTTACTAAAA ATTATCAAC CTGAGCCTGT CACAGGGGAA GAGAGATACA ACACTTGTT TTATGACCTG CACAGGGGAA TAATAATGTA GAATACATAT TTATACAAA TAATAATGTA GAATACATAT TTATACAAA TAATAATGTA GAATACATAT TTATGACTG CTTTCCAGAA TAGATACTAA TATTCTACAC CTTTGTAAAT TATGATAATA TTTTATATCCC CTTAGCCTAA GTCTTAGACA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG TAATCTAATT TATGACACA CTTTGTAAAT TATGATAATA TTTTTATATCCC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG TAATCTTATT TCTCTATTTC CTCCATTATT TCTCTATTTC CTCCATTATT TCTCTATTTC CTCCATTATT TCTCCATTATT TCTCCATTATT TCTCCATTATT TCTCCATTATT TTTTGAGTGG ACACTTAGA GCCCCAGGC ACCACGGAC ACCTCTAAA GACAGTTAGG ACACATAAC GCCCCAGAC AAATCCCTCA GCCCCAGCC ACCAGGAC ACACTCTAAA GCCCCCAGC AAATCCCTCA GCCCCAGCC ACCAGGAC ACACATAATA GCCCCCAGC ACCCCAGC ACCCCCAGC ACCCCCCAGC ACCCCCAGC ACCCCCCAGC ACCCCCAGC ACCCCCAGC ACCCCCAGC ACCCCCAGC ACCCCCCCC	6616 6676 6736 6796 6856 6916 6976 7036 7156 7216 7336 7396 7516 7536 7636 7756
40 45	AAAGTGAATA CTTACTAAAA ATTATCAAAC CTCTTACCTA TTGTGATAAT CACAGGGGAA ACACTTGTT TTATACAAA ATAAATGTA ACAATCAGTC TTTATACAAA TAATAATGTA ACAATCAGTC TTTATACAAA TAATAATGTA ACAATCAGTC TTTATACAAA TAATAATGTA ACAATCAGTC CTTTCCAGAA TGAGTTCTC TTATACACA TTTATACAAA TAATAATGTA ATTATCAACA CTTTCCAGAA TGAGTTCTC TTTGTAAAT TATGATAATA TTTTATATCCC CTTAGCCTAA GTCTTAGACA CTTTGTAAAT TATGATAATA TTTTATATCCC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTTGTTT TCTTATTTC TCTCATTTC CTCCATTATT TTTTGAGCTC CCACCAGTC CCCACCAGAC ACTTGAGAGAT GCCAGGGATG CTTACAAATT GGCAATACA GCCCCAGAC AAATCCCTC CCCACTGAAA GCCCCCAGAC AAATCCCTCA AAATCCCTCA GCAATAAA ATATAATCC CCCACTGAAC GCCCCAGAC AAATCCCTCA AAATCCCCC CCCACTGAC AAATCCCCC AACAGTACA GCCCCAGAC AAATCCCCCC AACAGATAC GCCCCAGAC AACTCTCAAA ATATAATCC CCCACTGAAC AACCATTCTA GCCATCACA CCCCAGCC AACAGATAC GCCCCAGAC AACTCCCCCA AACAGATAC GCCCCAGAC AACCCCCCAGAC AACCCCCAGAC AACCCCCCAGC AACCCCCCCC	6616 6676 6736 6796 6856 6916 6976 7036 70156 7216 7216 7336 7456 7556 7556 7756 7756 7756
40 45	AAAGTGAATA CTTACTAAAA ATTATCAAAC CTGAGCCTGT CACAGGGGAA ACAGGGGAA ACACTTGTT TTATACAAA ATAAATGTA ACAATCAGTC TTTATACAAA TAATAATGTA ACAATCAGTC TTTATACAAA TAATAATGTA AAAAAAAT TTTCTACAC CTTTGTAAAT ATTGATAATA ATTTCTACAC CTTTGTAAAT TATGATAATA TATGATAATA TTTTATACCC CTTAGCCTAA GTCTTAGACA CTTTGTAAAT TATGATAATA TTTTATATCCC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTTTTT TCTTATTT CTAGTTT TCTCATTTC CTCCATTATT CTCATTTT CTCATTTT CTCATTTT CTCATTTT CTCATTTC CTCCATTATT CTCATTATT TCTCATTTC CTCCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATC CTCCATTATA AGACCCC CCCCCAGTC CCCCCCAGAC ACTTGGAGC AACCACTCTAAA GACAGTTAGC AACAGATACA GCCCCCCAGAC AATCCCTCA AATCCCTCA AACAGATACA GCCCCCAGAC AACTCCTCAAA ATATAATCCA AGCTGAGCC TTCAGGTGAC CCCCCGGAC AACTCCTCAAA ATATAATCCA AGCTGAGCG CCCCCGGAC AACTCCTCAAA ATATAATCCA AGCTGAGCG CCCCCGGAC AACTCCTCAAA ATATAATCCA CCCCAACTAA AATTCCCTC AGCCTGAAG AACCCTCTAAA ATATAATCCA CCCCAACTAA AATTCCCTC TCCAACTAAC AACACTAATTA GAAGGGAAC CCCCCAGAC AACCCCCAGAC AACCCCCCAGAC AACCCCCCCAGAC AACCCCCCAGAC AACCCCCCCAGAC AACCCCCCCAGAC AACCCCCCCAGAC AACCCCCCCC	6616 6676 6736 6796 6856 6916 7036 7036 7216 7276 7336 7456 7576 7636 77516 77
40 45	AAAGTGAATA CTACAGAGAA AACACTTGTT CTAGCCTGT CACAGGGAA AGAGAGATACA ACACTTGTT TTATACAAA TAATACAAA TAATACATAT TATACAAA TAATACATAT TATACAAA TAATACATAT TATACAAA TAATACATAT TATCTACAC CTTTCCAGAA TAGCTTCTC TATGACATC CTTTGTAAAT TATGATAATA TATGATAATA TATGATAATA TATCTACAC CTTTGTAAAT TATGATAATA TATGATATA TAGCTAGTTC TAGTTGTTT TTTTTAATCC CTTAGCTTAA GAATAAAAGT TATGAGATCA GCCAGGTTT GAATAAAAAGT TATGAGATCA AGCCAGGTAT TACCTATTTC CTCTATTTC CTCTATTTC CTCTATTTC CTCTATTTC CTCCATTATT TCCCATTATT TCCCATTATT TCCCATTATT TCCCATTATT TCCCATTATT TCCCATTATA GCCAGGATG TTTTTGAGGA GCCAGGATG CCCACCAGAC AGAAAAAAA GCCACCAGAC AAATCCCTCA GCATAAGACT TTCAGGTGAC ACACAATTAA CCACAAATTAA CCACAATTAA CCACAAATTAA CCACA	6616 6676 6736 6796 68516 6976 7036 7036 7216 7216 7336 7456 7557 7636 7696 7756 7816 7936 7996
40 45 50	AAAGTGAATA CTACAGAGAA AACACTTGTT CTAGCCTGT CACAGGGAA AGAGAGATACA ACACTTGTT TTATACAAA TAATACAAA TAATACATAT TATACAAA TAATACATAT TATACAAA TAATACATAT TATACAAA TAATACATAT TATCTACAC CTTTCCAGAA TAGCTTCTC TATGACATC CTTTGTAAAT TATGATAATA TATGATAATA TATGATAATA TATCTACAC CTTTGTAAAT TATGATAATA TATGATATA TAGCTAGTTC TAGTTGTTT TTTTTAATCC CTTAGCTTAA GAATAAAAGT TATGAGATCA GCCAGGTTT GAATAAAAAGT TATGAGATCA AGCCAGGTAT TACCTATTTC CTCTATTTC CTCTATTTC CTCTATTTC CTCTATTTC CTCCATTATT TCCCATTATT TCCCATTATT TCCCATTATT TCCCATTATT TCCCATTATT TCCCATTATA GCCAGGATG TTTTTGAGGA GCCAGGATG CCCACCAGAC AGAAAAAAA GCCACCAGAC AAATCCCTCA GCATAAGACT TTCAGGTGAC ACACAATTAA CCACAAATTAA CCACAATTAA CCACAAATTAA CCACA	6616 6676 6776 6776 6856 6856 6976 7036 7036 7276 7336 7456 7576 7636 7876 7876 7876 7876 7876 7876 78
40 45	AAAGTGAATA CTTACTAAAA ATTATCAAAC CTGAGCCTGT CACAGGGGAA ACAGGGGAA ACACTTGTT TTATACAAA ATAAATGTA ACAATCAGTC TTTATACAAA TAATAATGTA ACAATCAGTC TTTATACAAA TAATAATGTA AAAAAAAT TTTCTACAC CTTTGTAAAT ATTGATAATA ATTTCTACAC CTTTGTAAAT TATGATAATA TATGATAATA TTTTATACCC CTTAGCCTAA GTCTTAGACA CTTTGTAAAT TATGATAATA TTTTATATCCC CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTTTTT TCTTATTT CTAGTTT TCTCATTTC CTCCATTATT CTCATTTT CTCATTTT CTCATTTT CTCATTTT CTCATTTC CTCCATTATT CTCATTATT TCTCATTTC CTCCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATT CTCATTATC CTCCATTATA AGACCCC CCCCCAGTC CCCCCCAGAC ACTTGGAGC AACCACTCTAAA GACAGTTAGC AACAGATACA GCCCCCCAGAC AATCCCTCA AATCCCTCA AACAGATACA GCCCCCAGAC AACTCCTCAAA ATATAATCCA AGCTGAGCC TTCAGGTGAC CCCCCGGAC AACTCCTCAAA ATATAATCCA AGCTGAGCG CCCCCGGAC AACTCCTCAAA ATATAATCCA AGCTGAGCG CCCCCGGAC AACTCCTCAAA ATATAATCCA CCCCAACTAA AATTCCCTC AGCCTGAAG AACCCTCTAAA ATATAATCCA CCCCAACTAA AATTCCCTC TCCAACTAAC AACACTAATTA GAAGGGAAC CCCCCAGAC AACCCCCAGAC AACCCCCCAGAC AACCCCCCCAGAC AACCCCCCAGAC AACCCCCCCAGAC AACCCCCCCAGAC AACCCCCCCAGAC AACCCCCCCC	6616 6676 6736 6796 68516 6976 7036 7036 7216 7216 7336 7456 7557 7636 7696 7756 7816 7936 7996

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GGGCGGGGG TGGCTGGAAG AGATCTGTGT AAATGAGGGA ATCTGACATT TAAGCTTCAT CAGCATCATA GCAAATCTGC TTCTGGAAGG AACTCAATAA ATATTAGTTG GAGGGGGGGA
                                                                                                                 8236
                                                                                                                 8296
           GAGAGTGAGG GGTGGACTAG GACCAGTTTT AGCCCTTGTC TTTAATCCCT TTTCCTGCCA
                                                                                                                 8356
           8416
           TCTCACTTGA GATCAGGAGT TCAAGACCAG CCTGGCCAGC ATGGCGATAC TCTGTCTCTA
                                                                                                                 8536
           CTAAAAAAA TACAAAAATT AGCCAGGCAT GGTGGCATGC ACCTGTAATC CCAGCTACTC
                                                                                                                 8596
           GTGAGCCTGA GGCAGAAGAA TCGCTTGAAA CCAGGAGGTG TAGGCTGCAG TGAGCTGAGA
                                                                                                                 8656
           TCGCACCACT GCACTCCAGC CTGGGCGACA GAATGAGACT TTGTCTCAAA AAAAGAAAAA GATACAACAG GCTACCCTTA TGTGCTCACC TTTCACTGTT GATTACTAGC TATAAAGTCC
                                                                                                                 8716
10
                                                                                                                 8776
           TATAAAGTTC TTTGGTCAAG AACCTTGACA ACACTAAGAG GGATTTGCTT TGAGAGGTTA
                                                                                                                 8836
           CTGTCAGAGT CTGTTTCATA TATATACATA TACATGTATA TATGTATCTA TATCCAGGCT
                                                                                                                 8896
           TGGCCAGGGT TCCCTCAGAC TTTCCAGTGC ACTTGGGAGA TGTTAGGTCA ATATCAACTT
          8956
                                                                                                                 9016
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                                                                                                                 9256
                                                                                                                 9316
                                                                                                                 9376
           GGGTTCACGC CATTCTCCTG CCTCACCCTC CCAAGCAGCT GGGACTACAG GCGCCTGCCA
CCATGCCCAG CTAATTTTTT GTATTTTTAG TAGAGACGGG GTTTCACCGT GTTAGCCAGG
                                                                                                                 9436
20
                                                                                                                 9496
           ATGGTCTCGA TCTCCTGAAC TTGTGATCCG CCCGCCTCAG CCTCCCAAAG TGCTGGGATT
          ATGGTCTCGA TCTCCTGAAC TTGTGATCCG CCCGCCTCAG CCTCCCAAAG TGCTGGGATT
ACAGGCGTGA GCCATCGCAC CCGGCTCAAC TGTAACTTTC TATACTGGTT CATCTTCCCC
TGTAATGTTA CTAGAGCTTT TGAAGTTTTG GCTATGGGTT ATTTCTCATT TATACATTAG
ATTTCAGATT AGTTCCAAAT TGATGCCCAC AGCTTAGGGT CTCTTCCTAA ATTGTATATT
GTAGACAGCT GCAGAAGTGG GTGCCAATAG GGGAACTAGT TTATACTTTC ATCAACTTAG
GACCCACACT TGTTGATAAA GAACAAAGGT CAAGAGTTAT GACTACTGAT TCCACAACTG
ATTGAGAAGT TGGAGATAAC CCCGTGACCT CTGCCATCCA GAGTCTTTCA GGCATCTTTG
AAGGATGAAG AAATGCTATT TTAATTTTG AGGTTCCTCA ATCAGTGCTT AGGATCATGA
                                                                                                                 9556
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           GAATCTGTGC TGCCATGAGG CCAAAATTAA GTCCAAAACA TCTACTGGTT CCAGGATTAA 10036
CATGGAAGAA CCTTAGGTGG TGCCCACATG TTCTGATCCA TCCTGCAAAA TAGACATGCT 10096
GCACTAACAG GAAAAGTGCA GGCAGCACTA CCAGTTGGAT AACCTGCAAG ATTATAGTTT 10156
CAAGTAATCT AACCATTTCT CACAAGGCCC TATTCTGTGA CTGAAACATA CAAGAATCTG 10216
30
           CATTTGGCCT TCTAAGGCAG GGCCCAGCCA AGGAGACCAT ATTCAGGACA GAAATTCAAG 10276
           ACTACTATEG ACTEGRACIA COCCAGGE AAGACAGACT CAACAATTAT TEGGTCTATT 10396
AATACAGCAG GCTTACACAG GAACCCAGGG CCTAGCCCTA CAACAATTAT TEGGTCTATT 10396
CACTGTAAGT TTTAATTTCA GGCTCCACTG AAAGAGTAAG CTAAGATTCC TEGCACTTTC 10456
TGTCTCTCTC ACAGTTEGCT CAGAAATGAG AACTEGTCAG GCCAGGCATG GTEGCTTACA 10516
35
           CCTGGAATCC CAGCACTTTG GGAGGCCGAA GTGGGAGGGT CACTTGAGGC CAGGAGTTCA 10576
           GGACCAGCTT AGGCAACAAA GTGAGATACC CCCTGACCCC TTCTCTACAA AAATAAATTT 10636
TAAAAATTAG CCAAATGTGG TGGTGTATAC TTACAGTCCC AGCTACTCAG GAGGCTGAGG 10696
           CAGGGGGATT GCTTGAGCCC AGGAATTCAA GGCTGCAGTG AGCTATGATT TCACCACTGC 10756
ACTTCTGGCT GGGCAACAGA GCGAGACCCT GTCTCAAAGC AAAAAGAAAA AGAAACTAGA 10816
40
           ACTAGCCTAA GTTTGTGGGA GGAGGTCATC ATCGTCTTTA GCCGTGAATG GTTATTATAG 10876
AGGACAGAAA TTGACATTAG CCCAAAAAGC TTGTGGTCTT TGCTGGAACT CTACTTAATC 10936
           TTGAGCAAAT GTGGACACCA CTCAATGGGA GAGGAGAAA GTAAGCTGTT TGATGTATAG 10996
           GGGAAAACTA GAGGCCTGGA ACTGAATATG CATCCCATGA CAGGGAGAAT AGGAGATTCG 11056
           GAGTTAAGAA GGAGAGGAGG TCAGTACTGC TGTTCAGAGA TTTTTTTTAT GTAACTCTTG 11116
           AGAAGCAAAA CTACTTTTGT TCTGTTTGGT AATATACTTC AAAACAAACT TCATATATTC 11176
           AAATTGTTCA TGTCCTGAAA TAATTAGGTA ATGTTTTTTT CTCTATAG GAA ATG AAT
                                                                                           Glu Met Asn
                                                                                           85
           CCT CCT GAT AAC ATC AAG GAT ACA AAA AGT GAC ATC ATA TTC TTT CAG
                                                                                                               11281
           Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Glu
50
                       90
                                                     95
                                                                                   100
           AGA AGT GTC CCA GGA CAT GAT AAT AAG ATG CAA TTT GAA TCT TCA TCA
                                                                                                               11329
           Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser
                                               110
                                                                             115
           TAC GAA GGA TAC TTT CTA GCT TGT GAA AAA GAG AGA GAC CTT TTT AAA
                                                                                                               11377
           Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys
55
                                         125
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	CTC ATT TTG AAA AAA GAG GAT GAA TTG GGG GAT AGA TCT ATA ATG TTC 11 Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe 140 145 150	425											
5	3 CT CTT C3 3 3 3 C C3 3 C3 C T3 CCT3 TTT 3 3 3 TTTT C3 TC C	464											
	(19) INFORMATION FOR SEQ ID NO: 18:												
10	(i)SEQUENCE CHARACTERISTICS: (A)LENGTH: 471 base pairs (B)TYPE: nucleic acid (C)STRANDEDNESS: double (D)TOPOLOGY: linear												
15	(ii) MOLECULE TYPE: cDNA to mRNA												
	(vi)ORIGINAL SOURCE: (A)ORGANISM: mouse (G)CELL TYPE: liver												
20	(ix)FEATURE: (A)NAME/KEY: mat peptide (B)LOCATION: 1471 (C)IDENTIFICATION METHOD: S												
25	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:												
	AAC TTT GGC CGA CTT CAC TGT ACA ACC GCA GTA ATA CGG AAT ATA AAT Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn 1 1 15 15	В											
30	GAC CAA GTT CTC TTC GTT GAC AAA AGA CAG CCT GTG TTC GAG GAT ATG Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met 20 25 30	5											
	ACT GAT ATT GAT CAA AGT GCC AGT GAA CCC CAG ACC AGA CTG ATA ATA Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile 35	1											
35	TAC ATG TAC AAA GAC AGT GAA GTA AGA GGA CTG GCT GTG ACC CTC TCT 192 Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser 50 55 60	2											
	GTG AAG GAT AGT AAA ATG TCT ACC CTC TCC TGT AAG AAC AAG ATC ATT 240 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile)											
40	65 70 75 80 TCC TTT GAG GAA ATG GAT CCA CCT GAA AAT ATT GAT GAT ATA CAA AGT 288	3											
	Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser 85 90 95												
45	GAT CTC ATA TTC TTT CAG AAA CGT GTT CCA GGA CAC AAC AAG ATG GAG 336 Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu 100 105 110	5											
~	TTT GAA TCT TCA CTG TAT GAA GGA CAC TTT CTT GCT TGC CAA AAG GAA Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu 115 120 125	ŀ											
50	GAT GAT GCT TTC AAA CTC ATT CTG AAA AAA AAG GAT GAA AAT GGG GAT Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp 130 135 140	2											
50	AAA TCT GTA ATG TTC ACT CTC ACT AAC TTA CAT CAA AGT Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser 145 150 155	-											
55	(20) INFORMATION FOR SEQ ID NO: 19:												
3 -	(i) SEQUENCE CHARACTERISTICS:												

- (A) LENGTH: 9 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (v) FRAGMENT TYPE: N-terminal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Asn Phe Gly Arg Leu His Cys Thr Thr

- (21) INFORMATION FOR SEQ ID NO: 20:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids(B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide 20 .

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 20 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile
50 60 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 70 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 100 105 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 115 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu

140

- (22) INFORMATION FOR SEQ ID NO: 21:
 - (i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 157 amino acids(B) TYPE: amino acid

135

Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 25 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile

Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 50 60 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 70 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 85 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 105 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu
115 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150

(23) INFORMATION FOR SEQ ID NO: 22:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 (B) TYPE: amino acid

 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 20 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 50 55 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 65 70 75 80 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 85 90 95 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 100 105 110 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 130 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150

- (24) INFORMATION FOR SEQ ID NO: 23:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:
- Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 55 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp

- Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 50 55 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile 70 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 85 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 10 100 105 110 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 120 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 15 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150 (25) INFORMATION FOR SEQ ID NO: 24: (i) SEQUENCE CHARACTERISTICS: 20 (A) LENGTH: 157 amino acids

 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 25 30 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 35 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 50 55 60 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ser Glu Asn Lys Ile 70 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 90 95 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 115 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 130 135 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150

- (26) INFORMATION FOR SEQ ID NO: 25:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids (B) TYPE: amino acid

 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:
- Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 55

- Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 20 25 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 50 55 60 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 10 85 90 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys 100 105 110 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu 115 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 130 140 15 140 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 150
 - (27) INFORMATION FOR SEQ ID NO: 26:

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- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:
- Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn 10 15 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp 20 25 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile 35 40 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile 55 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile 70 75 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys 85 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu 115 120 125 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu 135 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp 145 150
 - (28) INFORMATION FOR SEQ ID NO: 27:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27: 55

Asn Phe Gly Arg Leu His Ala Thr Thr Ala Val Ile Arg Asn Ile Asn 10 Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met 20 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile 40 Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser 55 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile 70 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser 85 90 95 Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu 100 105 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu 115 120 125 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp 135 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser 145 150 155

- (29) INFORMATION FOR SEQ ID NO: 28:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 157 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
 - (ii) MOLECULE TYPE: peptide
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn 10 Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met 20 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser 50 55 60 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile 70 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser 85 Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu 100 105 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Ser Gln Lys Glu 115 120 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp 135 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser 150

Claims

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- 1. An osteoclastgenic inhibitory agent, which comprises an interleukin-18 or its functional equivalent.
- 2. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3 as partial amino acid sequences.
 - 3. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequences of SEQ ID NO: 4

and SEQ ID NO: 5 as partial amino acid sequences.

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- 4. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequence of SEQ ID NO: 6.
- 5 5. The inhibitory agent of claim 1, wherein said interleukin-18 is human origin.
 - 6. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequence of SEQ ID NO: 7.
- 7. The inhibitory agent of claim 1, which is a therapeutic agent for osteoclast-related diseases.
 - 8. The inhibitory agent of claim 1, which contains a protein, buffer, or saccharide as a stabilizer.
 - 9. The inhibitory agent of claim 1, which is in the form of a liquid, paste, or solid.
- 15. The inhibitory agent of claim 1, which contains 0.000002-100 w/w % of said interleukin-18.
 - 11. An inhibitory agent as defined in any preceding claim, for use as a pharmaceutical.
- 12. Use of an inhibitory agent as defined in any of claims 1-10 for the preparation of a medicament effective for treating and/or preventing osteoclast-related diseases.

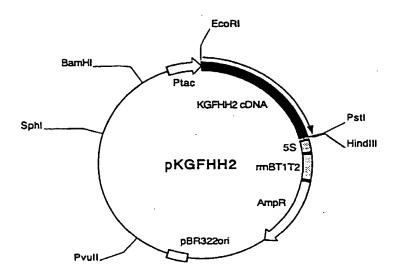


FIG. 1

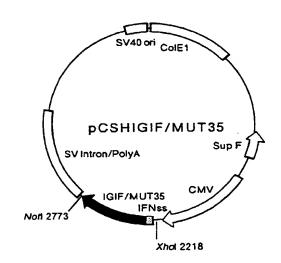


FIG. 2

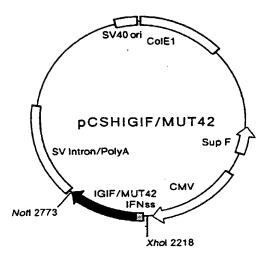


FIG. 3

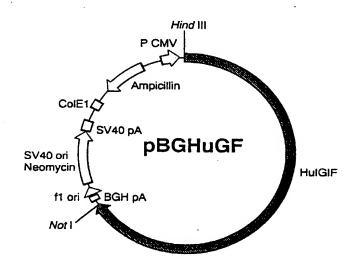


FIG. 4

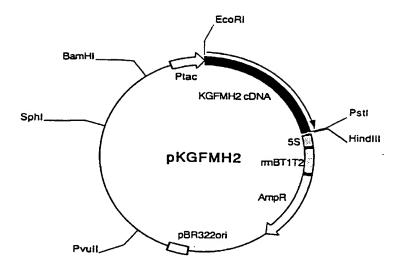


FIG. 5